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Occurrence of a Lotic Breeding *Hynobius* Salamander (Amphibia, Urodela) on Kamishima of the Amakusa Islands, Japan

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Abstract: We found a salamander of the genus *Hynobius* on Kamishima of the Amakusa Islands, Japan, which is new to this island group. From the characteristics of larval habitat and morphology, this salamander is considered to be a lotic breeder. Until the discovery of this species, only a lentic breeding species (*Hynobius nebulosus*) was known from the Amakusa Islands. Morphologically the present salamander is well differentiated from other lotic breeding *Hynobius* of nearby regions and is closest to the southernmost population of *H. boulengeri* from the Osumi Peninsula among the species or populations examined. In order to determine its taxonomic position, however, genetic analyses using a larger number of comparative specimens are needed. The biogeographic significance of the present finding is briefly discussed.

Key words: Hynobiidae; *Hynobius*; the Amakusa Islands; Lotic breeding; Biogeography

INTRODUCTION

The Amakusa Islands is located ca. 15 km west of Kyushu, Japan, and consists of two large islands, Kamishima (225 km^2 in area) and Shimoshima (574 km^2 in area), and approximately 120 much smaller islets (Fig. 1). Islands of this group are largely low in altitude, with a peak of Mt. Kuratake on Kamishima Island (682 m asl) representing the highest point.

A total of 11 native amphibians (two species of Urodela [*Hynobius nebulosus* and *Cynops pyrrhogater*], and nine of Anura [*Bufo japanicus japonicus*, *Hyla japonica*, *Rana tagoi*, *R. japonica*, *R. nigromaculata*, *R. limnocharis*, *R. rugosa*, *Buergeria buergeri*, and *Rhacophorus schlegelii*]) and one introduced frog (*Rana catesbeiana*) have been recorded from the Amakusa Islands (Okochi and Hayashi, 1992; Maeda and Matsui, 1999; Sakamoto, unpublished data). Because all these species are also very common in the western part of the main island of Kyushu facing the islands, little attention has been paid to the amphibian

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FIG. 1. A map of the Amakusa Islands and the Kyushu Main Island, Japan, showing sampling localities of hynobiid species used in this study. Coarsely dotted and darkened areas represent areas >500 m and 1000 m asl, respectively.

fauna of this island group until now.

Recently, we collected specimens of a *Hynobius* salamander along a small stream of Kamishima Island. The specimens resembled *H. naevius* at a glance, but were not easy to identify. However, this form was obviously a lotic breeder (Sato, 1943), because it had a stout tail, mountain habitat, and stream dwelling larvae with claw-like cutaneous structures. Up to now, *H. nebulosus*, a lentic breeder, has been found in lowlands of Kamishima and Shimoshima Islands as mentioned above. This species possesses a compressed tail with yellowish streaks on its edges, and these morphological characteristics, along with its breeding in the still water, completely differentiate *H. nebulosus* from the salamander in question. From the southern part of Kyushu, three species of lotic breeding *Hynobius* salamanders, *H. naevius*, *H. boulengeri*, and *H. stejnegeri*, have been reported.

In order to assess the taxonomic status of this salamander, we investigated its natural history and compared its morphological characteristics with those of its putative relatives from the main island of Kyushu.

MATERIALS AND METHODS

The salamander was searched for on 7 April, 7 May, and 14 October 2002, along small streams on Kamishima Island, Kumamoto Prefecture (detailed locality information is not given for conservation reasons). Environmental conditions of the habitat were recorded, and salamanders collected were fully anesthetized and their livers were removed for genetic study. They were then fixed in 10% formalin before final preservation in 70% ethanol.

Four populations of three lotic breeding *Hynobius* species from the central and southern parts of Kyushu Main Island were used for morphological comparisons—one representing *H. naevius*, another *H. stejnegeri*, and the remaining two (one from the Sobo-Katamuki Mountains and the other from Osumi Peninsula) *H. boulengeri* (Fig. 1). Of the three forms of *H. naevius* recognized by Sato et al. (1994) from Kyushu, we used one population of the southern Kyushu type (Sato et al., 1994) that is distributed close to the Amakusa Islands. See Appendix for current locations of voucher specimens.

The following seven morphometric characters were measured; SVL (snout–vent length, from tip of snout to anterior angle of cloaca), HL (head length, from tip of snout to gular fold), HLL (hind limb length), TAL (tail length), HW (head width, measured at jaw articulation), VTL (length of vomerine tooth series), and VTW (width of vomerine tooth series). Measurements were taken to the nearest 0.1 mm with dial calipers. Values of SVL and those of HL, HLL, TAL, HW in relation to SVL were compared among samples. The ratio of VTW to VTL (VTW/VTL), one of the key characters for species identification in this group (Sato, 1943), was also obtained and compared. The number of costal grooves (CG) was counted, and overlap of finger and toe tips (limb overlap, LO) when both forelimbs and hind limbs were adpressed along the body was

recorded as the number of costal folds between those tips. In this character, a plus value indicates overlap and a minus value indicates separation. We also counted the numbers of teeth on the vomerine (VTN), upper jaw (UJTN), and lower jaw (LJTN). Differences in SVL and ratio values among samples were statistically tested by Student's t-test and Mann-Whitney's U test, respectively. The definitions of characters followed Matsui (1987). The significance level was 95% in all these statistical tests.

To summarize the morphological relationships among populations studied, we performed canonical discriminant analyses (CDA) with the CANDISC procedure (SAS, 1990). In these analyses, we omitted TAL because not all individuals had intact tails.

Results

Habitat, life history, and morphology

The salamanders were collected in the daytime under fallen leaves and gravel along a small stream (maximum width = 3 m) on 7 April and 7 May 2002. The stream was surrounded by a forest of planted cedars (*Cryptomeria japonica*) and its banks were covered with native plant species. This type of environment was typical of the montane regions on Kamishima Island (Fig. 2A). The bottom of the stream was covered mainly with rocks (Fig. 2B). There were small waterfalls (ca. 1 m in height) near the place where we found salamanders.

Nine males, one female, and two juveniles were collected on 7 May 2002 from one locality. The testes and ovaries of these adults were atrophied. Fourteen larvae were also collected on 14 October 2002 in the moderately flowing stream along which metamorphs had been collected. These larvae were found at night near the banks of the stream.

Morphological data of the adults are summarized in Tables 1–3. Males were smaller in SVL and had relatively longer and stouter limbs than in the single female. In all adults, the head was moderately depressed and body was robust. Six of the ten adults had regenerated tails, and the intact tails were short and thick. The posterior half of the tail was keeled, moderately above and weakly below. The dorsum was brownish gray. The trunk had numerous silvery dots mainly on the lateral and ventral sides, with a few on the dorsal side (Fig. 3). The two juveniles collected in early May measured 32.7 and 33.4 mm in SVL. The

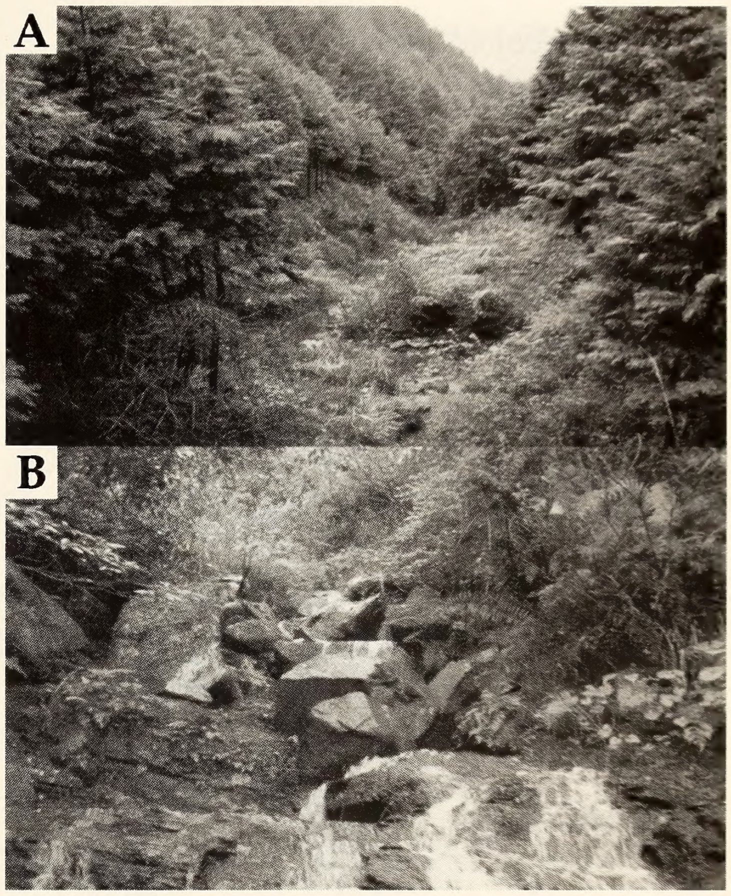

FIG. 2. (A) Overall view of the habitat of *Hynobius* sp. on Kamishima Island, showing the typical vegetation. (B) The stream where adults and larvae of *H.* sp. were found.

FIG. 3. An adult male of *Hynobius* sp. from Kamishima Island.

juveniles had more silvery dots over the whole body surface than the adults.

Fourteen larvae found in October had SVLs ranging from 22.2 to 28.5 ($\bar{x}=25.5$) mm. Five of these larvae were thought to have started metamorphosis because of obvious reductions in their gills and tail fins. All larvae possessed a claw-like dark cutaneous structure on each digital tip. The body was light brown in ground color marbled with dark grey.

Morphological comparisons

Because only one female specimen was available from Kamishima Island, we compared only male samples in detail. In SVL, males from Kamishima Island were significantly smaller than those of the species compared except for *H. boulengeri* from Osumi Peninsula (Table 1). Compared with the salamander from Kamishima Island, *H. boulengeri* from the Sobo-Katamuki Mountains had a relatively long head, while *H. boulengeri* from the Osumi Peninsula and *H. naevius* had relatively narrow heads. Also, *H. boulengeri* from the Sobo-Katamuki Mountains and *H. stejnegeri* had longer hind limbs

TABLE 1. Comparisons of SVL ($\bar{x}\pm2SE$, followed by ranges in parentheses, in mm) and ratio (in %) of each character dimension to SVL (medians, followed by ranges in parentheses) in five samples of the *Hynobius* salamanders examined.

	Sex	n	SVL	HL/SVL	HW/SVL	HLL/SVL	n**	TAL/SVL
Hynobius sp. from the Amakusa Island	M	9	72.1±3.4 (64.8–79.0)	23.8 (22.1–25.2)	18.6 (17.0–20.5)	27.3 (25.8–29.0)	3	66.0 (61.5–66.5)
	F	1	83.7	22.5	17.0	26.2	1	62.6
H. boulengeri from the Sobo-Katamuki Mts.	M	22	83.0±2.3* (73.6–92.4)	24.6* (22.8–26.5)	17.8 (16.3–20.0)	30.5* (28.9–33.2)	16	90.5* (82.6–100.6)
H. boulengeri from the Osumi Pen.	M	8	68.5±2.3 (64.4–73.6)	23.5 (21.3–25.0)	17.0* (15.2–18.6)	28.4 (25.1–30.5)	4	72.5 (69.4–75.1)
H. stejnegeri	M	19	80.4±1.9* (72.2–87.7)	23.7 (22.0–25.8)	18.1 (16.8–20.1)	29.7* (27.4–32.6)	15	84.3* (76.5–92.2)
H. naevius from the Sobo-Katamuki Mts.	M	7	58.0±3.2* (51.8–64.2)	23.9 (23.6–26.0)	17.4* (15.5–19.0)	29.5 (26.8–30.2)	6	62.5 (61.0–67.5)

* Significantly different from male *H.* sp. at $P<0.05$.
** Individuals with regenerated tail excluded.

TABLE 2. Variation in the numbers of costal grooves (CG) and costal folds between adpressed limbs (LO) in the five samples of the *Hynobius* salamanders examined.

			CG		LO							
	Sex	n	13	14	−3	−2.5	−2	−1.5	−1	−0.5	0	0.5
Hynobius sp. from the Amakusa Island	M	9	9		2	4	2	1				
	F	1	1		1							
H. boulengeri from the Sobo-Katamuki Mts.	M	22	11	11				4	8	4	5	1
H. boulengeri from the Osumi Pen.	M	8	6	2	2	1	3	1	1			
H. stejnegeri	M	19	12	7			6	1	7	3	1	1
H. naevius from the Sobo-Katamuki Mts.	M	7	5	2		1	4	2				

TABLE 3. Comparisons in shape of the vomerine tooth series (VTW/VTL: median), and the numbers ($\bar{x}\pm2SE$) of vomerine teeth (VTN), upper jaw teeth (UJTN), and lower jaw teeth (LJTN) among the five samples of the *Hynobius* salamanders examined. Figures in parentheses indicate ranges.

	Sex	n	VTW/VTL	VTN	UJTN	LJTN
Hynobius sp. from the Amakusa Island	M	9	1.05	58.2±3.2	83.1±3.4	78.9±2.9
			(0.89–1.32)	(51–65)	(77–92)	(72–85)
	F	1	1.13	60	93	86
H. boulengeri from the Sobo-Katamuki Mts.	M	22	1.23*	53.4±2.2*	84.9±2.3	80.8±2.7
			(1.00–1.50)	(44–63)	(74–97)	(66–92)
H. boulengeri from the Osumi Pen.	M	8	1.08	51.5±3.7*	69.9±2.6*	68.9±2.9*
			(0.93–1.19)	(42–57)	(63–75)	(62–74)
H. stejnegeri	M	19	1.07	47.0±2.2*	78.2±3.4	77.5±3.1
			(0.95–1.35)	(39–58)	(68–91)	(63–90)
H. naevius from the Sobo-Katamuki Mts.	M	7	0.84*	45.0±3.3*	65.1±3.6*	62.1±5.5*
			(0.77–0.92)	(39–50)	(58–72)	(52–75)

* Significantly different from male *H.* sp. at $P<0.05$.

in relation to SVL than the Kamishima salamander (Table 1). The tail length of males from Kamishima Island was significantly shorter than that of males of *H. boulengeri* from the Sobo-Katamuki Mountains and *H. stejnegeri*.

The salamander from Kamishima Island consistently had 13 costal grooves, whereas specimens of each of the other populations examined had either 13 or 14 grooves (Table 2). The frequency of occurrence of 14 grooves was especially high in *H. boulengeri* from the Osumi Peninsula and *H. stejnegeri*. In the salamander from Kamishima Island and *H. boulengeri* from the Osumi Peninsula, the limbs were more widely separated than in the other samples (Table 2).

The salamander from Kamishima Island had a V-shaped vomerine tooth series. The ratio of width to length of the series (VTW/VTL) was larger in the *H. boulengeri* population from the Sobo-Katamuki Mountains (i.e., it had a shallower vomerine tooth series) and smaller in *H. naevius* (i.e., it had a deeper series) than in the salamander from Kamishima Island (Table 3). The salamander from Kamishima Island had more vomerine teeth than the others. Likewise, the number of teeth in upper and lower jaw was greater in the Kamishima Island salamander than in *H. boulengeri* from the Osumi Peninsula and *H. naevius* (Table 3).

TABLE 4. Standardized coefficients for the first three canonical axes (CANs 1–3) of variation of characters.

Characters	CAN1	CAN2	CAN3
SVL	−0.392	−0.842	0.084
HL	0.456	1.022	0.596
HW	0.595	−2.494	0.848
HLL	2.382	0.658	−2.977
VTW	0.853	1.538	1.578
VTL	−0.665	0.029	0.148
Proportion	0.874	0.078	0.036

Standardized coefficients of the six measurements calculated for the first three canonical axes (CANs 1–3) and proportions for these axes are presented in Table 4. Cumulative proportions indicate that CANs 1–3 account for 98.8% of the total variation. The first and second canonical axes account for 87.4% and 7.8% of the total variation, respectively. The high positive coefficient value of HLL was obtained in CAN 1 axis,

the high negative value of HW in CAN 2, and high negative value of HLL in CAN 3, and these were the main discriminanting features. The value of VTW was the second contribution for discrimination in all the three axes.

Figure 4 shows two dimensional plots of the first three canonical variables. The salamander from Kamishima Island largely overlapped *H. boulengeri* from the Osumi Peninsula on the first canonical axis. However, on the second and third axes, the two populations tended to be separated from each other.

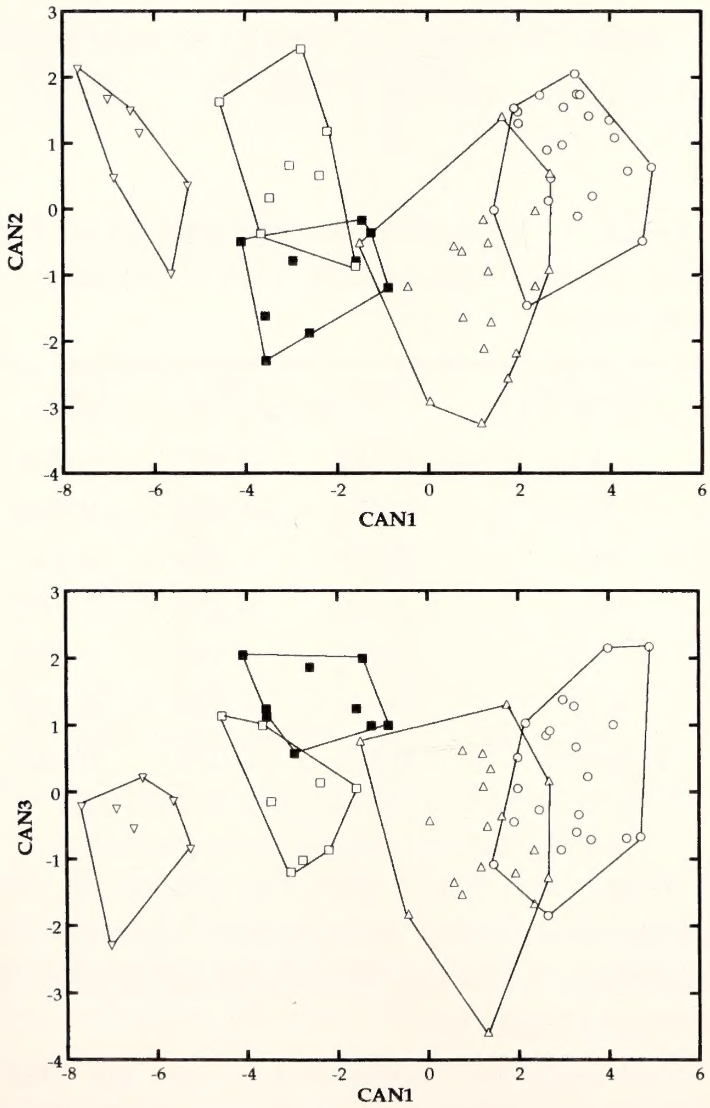

FIG. 4. Two dimensional plots of the first (CAN1) versus second (CAN2) canonical variables (upper) and the first versus third (CAN3) canonical variables (lower). Closed squares, open circles, open squares, open triangles, and reversed open triangles represent scores for specimens of *Hynobius* sp. on Kamishima Island, *H. boulengeri* from Sobo-Katamuki Mountains, Osumi Peninsula, *H. stejnegeri*, and *H. naevius*, respectively.

DISCUSSION

Natural history

The habitat and external morphology of larvae and adults of the salamander from Kamishima of the Amakusa Islands indicate that this is a lotic breeder (*H. naevius* group of Sato [1943]). The Kamishima salamander seems to breed before May and then stay near the breeding stream. The most important aspects of its natural history such as breeding season, oviposition site characteristics, clutch size, egg diameter, egg sac shape, hatching time, and size of larvae at hatching are totally unknown.

Some larvae seem to start metamorphosis before mid October, and the average size at metamorphosis is estimated as approximately 26 mm in SVL. However, some other larvae may overwinter and metamorphose at a larger size in the following spring or summer, as is usual with other lotic breeding *Hynobius* species (Misawa and Matsui, 1997). Estimated size at metamorphosis (ca. 26 mm) is smaller than that of *H. boulengeri* from the Sobo-Katamuki Mountains and *H. stejnegeri* (ca. 35–50 mm in SVL: Sato, 1954; Nishikawa et al., unpublished), but is similar to that of *H. boulengeri* from the Osumi Peninsula (ca. 25–35 mm: Sato, 2003; Nishikawa et al., unpublished). In *H. boulengeri*, geographically variable size at metamorphosis is thought to induce great variation in adult SVL (Nishikawa et al., 2001).

The two juveniles collected in May were slightly larger than older larvae collected in October, and thus seem to have metamorphosed before winter and increased in size. Sexual maturation seems to be attained at about 60–70 mm in SVL in males, and possibly at a larger size in females.

Taxonomic status

The color pattern of the salamander from the Kamishima Island, characterized by numerous silvery dots scattered on the brownish gray body, is shared with some individuals of *H. boulengeri* from the Osumi Peninsula. Such a pattern is also similar to that of *H. naevius*,

although silvery markings are more developed in this species. We found claw-like structures on the larval digital tips of the Amakusa salamander, which is common to populations of *H. boulengeri* from the Kyushu Main Island and *H. stejnegeri* but is absent in *H. naevius* (Sato, 1943; Sato, 1954, 2003).

In SVL of adult males, H. sp. from Kamishima Island is smaller than *H. boulengeri* from the Sobo-Katamuki Mountains and *H. stejnegeri*, and is larger than the *H. naevius* we examined. The present salamander is most similar to *H. boulengeri* from the Osumi Peninsula but differs from it by a relatively wider head and larger numbers of vomerine and jaw teeth. However, the sample size currently available is not sufficient to make a taxonomic decision now, and further morphological analysis based on additional specimens, especially of females, and genetic analysis are needed to determine the taxonomic status of this salamander.

Biogeographic implications

In the past, the Amakusa Islands may have experienced frequent connection to and separation from the Kyushu Main Island through the Yatsushiro Sea in between, which is very narrow and shallow (only 15 km wide and mainly 25–55 m deep currently). Occurrence of one of the largest pyroclastic flows from the Aso volcano (ca. 90000 yBP) found in the Amakusa Islands demonstrates the relatively recent land connection of this island group with the Kyushu Main Island (Machida, 2001), and can explain the high similarity of the batrachian fauna between them.

The presence of two *Hynobius* species, *H. nebulosus* and *H.* sp., on Kamishima of the Amakusa Islands is ecologically very interesting because, in contrast to relatively large areas of the Main Islands of Japan, where more than one species occur, no more than one species is known from other small peripheral islands (e.g., only *H. okiensis* on Oki Island, *H. tsuensis* on Tsushima Island, and *H. nigrescens* on Sado Island). This is probably due to the small carrying capacities of these other islands. The exceptional coexistence of two *Hynobius* species on Kamishima Island may be partially attributable to their habitat segregation, with *H. nebulosus* occupying a niche in the lowlands, and *H.* sp. in the mountains. The relatively short period of isolation between the Amakusa Islands and the Kyushu Main Island might also be responsible for the current coexistence of these two species.

Conservation

Among the salamanders examined in this study, *H. boulengeri* from a limited area on the Sobo-Katamuki Mountains is protected by Oita Prefecture and *H. stejnegeri* from Kumamoto Prefecture is also locally protected by the prefectural government. These species are also listed in the Red Data Book by the Japan Ministry of Environment (Matsui, 2000). However, *H.* sp. from the Amakusa Islands, first reported here, and the population of *H. boulengeri* from the Osumi Peninsula are not protected at all. Because both of these two morphologically unique populations are surely facing artificial habitat destruction, measures for their conservation urgently need to be taken.

Acknowledgments

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APPENDIX

Sampling localities and catalogue number of specimens examined

Voucher specimens are stored in the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), S. Tanabe's private collection (T), S. Sato's private collection (ST), M. Sakamoto's private collection (SK), Osaka Museum of Natural History (OMNH), and the National Science Museum, Tokyo (NSMT).

Hynobius sp. from Kamishima of the Amakusa Islands: Kumamoto Prefecture (adults: KUHE 30332–30339, SK 4; juveniles: KUHE 30340, 30341)

H. boulengeri from the Sobo-Katamuki Mountains: Ume-cho, Oita Prefecture and Takachiho-cho, Miyazaki Prefecture (Adults: KUHE 18920, 21619, 22813, 22889, 24878, 24967, 25096, 26142, 27125, 27183, 27184, 28748–28752, 28753, 28754, 28755, 28756; two unnumbered ST specimens)

H. boulengeri from the Osumi Peninsula: Tashiro-cho and Koyama-cho, Kagoshima Prefecture (Adults: KUHE 18923, 22892, 24961, 24962, 28539; OMNH H108; NSMT H03664, H04534)

H. stejnegeri: Gokase-cho, Miyazaki Prefecture (Adults: KUHE 12983, 14955, 14956, 22815, 22817–22819, 26065–26068, 27156–27560; T 2537, 2808; one unnumbered ST specimen)

H. naevius: Ume-cho, Oita Prefecture (adults: KUHE 25098, 27383, 28846, four unnumbered ST specimens)

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A Fossorial Lizard with Forelimbs Only: Description of a New Genus and Species of Malagasy Skink (Reptilia: Squamata: Scincidae)

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Abstract: A new genus and species of fossorial scincine lizard is described from northeastern Madagascar. This species, having an elongated body and eyes covered by scales, lacking external ear openings and pigmentation throughout the body, resembles *Cryptoscincus* and *Voeltzkowia*. However it differs from these or any other scincid genera known to the present in having small but distinct forelimbs, each with four stout claws, and complete lack of hind limbs.

Key words: New genus; New species; Scincidae; Madagascar; Fossorial; Limb reduction

INTRODUCTION

Seven endemic genera of the subfamily Scincinae (Squamata, Scincidae) are known from Madagascar, and most of them consist of fossorial or semifossorial species (Angel, 1924; Brygoo, 1979, 1980a–d, 1981a–c, 1984a–d, 1985; Mocquad, 1909). Recent herpetological surveys on Madagscar led to discoveries of many new species (Raxworthy and Nussbaum, 1993; Nussbaum and Raxworthy, 1995; Andreone and Greer, 2002; Sakata and Hikida, 2003). Due to their largely secretive habits, however, many species of these and related genera are thought to remain undiscovered.

Ecological surveys, carried out by Japanese ornithologists and herpetologists in collaboration with their Malagasy counterparts in Ampijoroa, Ankarafantsika Strict Nature Reserve, northwestern Madagascar (Fig. 1), yielded two specimens of an apparently undescribed skink. This skink differs from any known scincids in having forelimbs only. We thus describe it as a new genus and species.

MATERIALS AND METHODS

The specimen was fixed with 10% formalin, preserved in 75% ethanol, and deposited in the Zoological Collection of the Kyoto University Museum (KUZ). The following measurements were taken with dial calipers and recorded to the nearest 0.1 mm: snout-vent length (SVL), tail length, head length (snout tip to posterior margin of parietals), snout length (snout tip to anterior corner of eye), head width (the widest point of temporal region), and midbody width. Vertebral characters were determined by radiographs (Softex M-60, Softex Co.).

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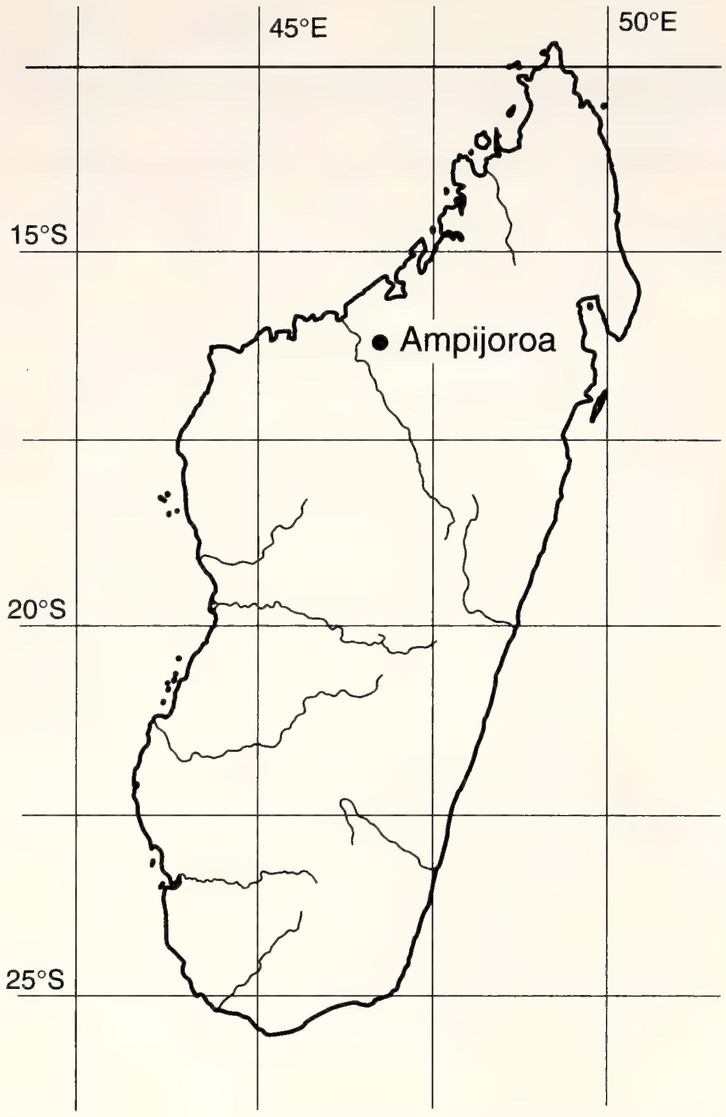

FIG. 1. Map of Madagascar, showing location of Ampijoroa, the type locality of *Sirenoscincus yamagishii* sp. nov.

We defined the scales covering the eyes as ocular(s). Terminology for the other characters follows Sakata and Hikida (2003).

Sirenoscincus yamagishii gen. et sp. nov.
Figs. 2 and 3

Holotype

KUZ R50922, mature female; Ampijoroa, Ankarafantsika Strict Nature Reserve, northwestern Madagascar (16 20'S, 46 48'E: Fig. 1), 100 m; collected by A. Mori, M. Hasegawa, and I. Ikeuchi, 7 November 1999.

Paratype

KUZ R50823, mature female, from the same locality as the holotype, collected by G. Razafindrakoto, as a "dead on the road" specimen, 11 November 1999.

Generic diagnosis

The new genus is a member of the subfamily Scincinae (Greer, 1970). It is distinguished from all other taxa in this group by the following combination of characters in derived states (polarity for each of these characters was inferred as relative to *Eumeces*, the putative primitive genus of the family: Greer and Broadley, 2000). Body highly elongated (SVL 11.6–14.0 times head length) with 53 presacral vertebrae; original tail slightly longer than SVL; snout pointed, lower jaw countersunk; neck indistinguishable externally; body scales smooth and transparent without pigmentation; 20 longitudinal scale rows at midbody. Forelimb small (approximately 4.7% of SVL) with indistinct fingers and four stout claws; no hind limbs, shallow groove in their position in other scincines; prefrontals absent; nasal sharply pointed, triangular, positioned at V-shaped notch of rostral; nostril positioned at anterior tip of nasal; frontonasal as large as frontal; frontoparietal absent; loreal single; supraoculars two; superciliaries and movable lower eyelid absent; eye covered with two oculars; supralabials six; infralabials five, anterior three higher than posterior two; external ear opening covered by scales, but small ear groove visible through transparent scale; postmental single.

Species diagnosis

The species diagnosis is the same as that for the genus.

Description of holotype

Adult female. Head much narrower than body; snout pointed; lower jaw countersunk; nostril anterolaterally oriented, visible from above; ear openings absent, small ear groove visible through transparent scales covering it; neck not distinct; body greatly elongated, with 53 presacral vertebrae; body and tail round in cross section; forelimbs small, with indistinct fingers and four stout claws; hind limbs absent.

Rostral scale large, overlapping nasals, supranasals and first supralabials; nasal sharply pointed and triangular, positioned in

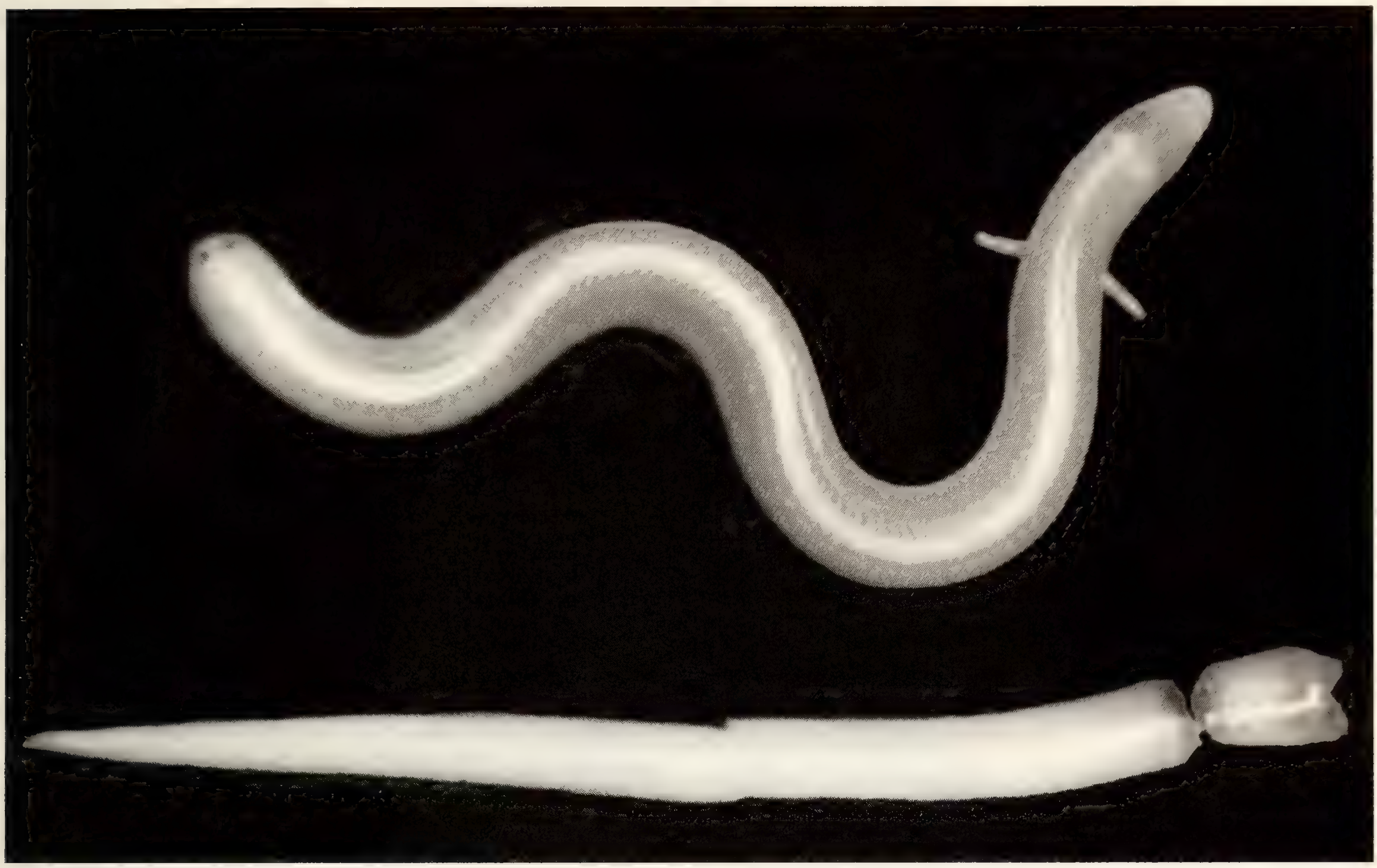

FIG. 2. Holotype of *Sirenoscincus yamagishii* sp. nov. (KUZ R50922) in life, with its autotomized tail.

the V-shaped notch of rostral, overlapping supranasal and first supralabial; nostril located in anterior tip of nasal, in contact with rostral; supranasals two, each overlapped by first supralabial, overlapping loreal and frontonasal, right overlapping left; frontonasal overlapped laterally by loreal, overlapping frontal and first supraocular posteriorly; prefrontals absent; frontal as large as frontonasal, overlapped laterally by first supraocular, overlapping interparietal and a pair of parietals posteriorly; frontoparietal absent; interparietal triangular, with two longer sides converging to a bluntly rounded apex posteriorly, overlapping parietals; transparent spot on interparietal absent; parietals two large, quadrangular, left overlapping right behind interparietal, each overlapped by first and second supraoculars, overlapping upper secondary temporal and first nuchals; two nuchals on left, one on right; supralabials six, three preorbital, one subocular and two postorbital; loreal single, quadrangular, as long as second supralabial, overlapped below by first and second supralabials, overlapping first supraocular and preocular posteriorly; preocular single, overlapped by second supralabial, overlapping first supraocular, first ocular, and third supralabial; presubocular absent; supraoculars two, overlapped by two oculars below, overlapping frontal and parietal above, and primary and upper secondary temporals posteriorly; oculars two, covering eye, overlapped by third supralabial, overlapping supraoculars, primary temporal and fourth supralabial; postsubocular absent; primary temporal one, overlapped by fourth supralabial, overlapping upper and lower secondary temporals and fifth supralabial; upper secondary temporal about half as long as parietal, overlapping lower secondary temporal, nuchal, and anteriormost scale of a lateral body scale row; lower secondary temporal overlapped by fifth supralabial, overlapping sixth supralabial and anteriormost scales belonging to lateral body scale rows; mental slightly larger than postmental, overlapping postmental and first infralabials; postmental overlapped by first infralabials, overlapping the first pair of chin shields; three pair of chin shields, first pair in contact, second separated

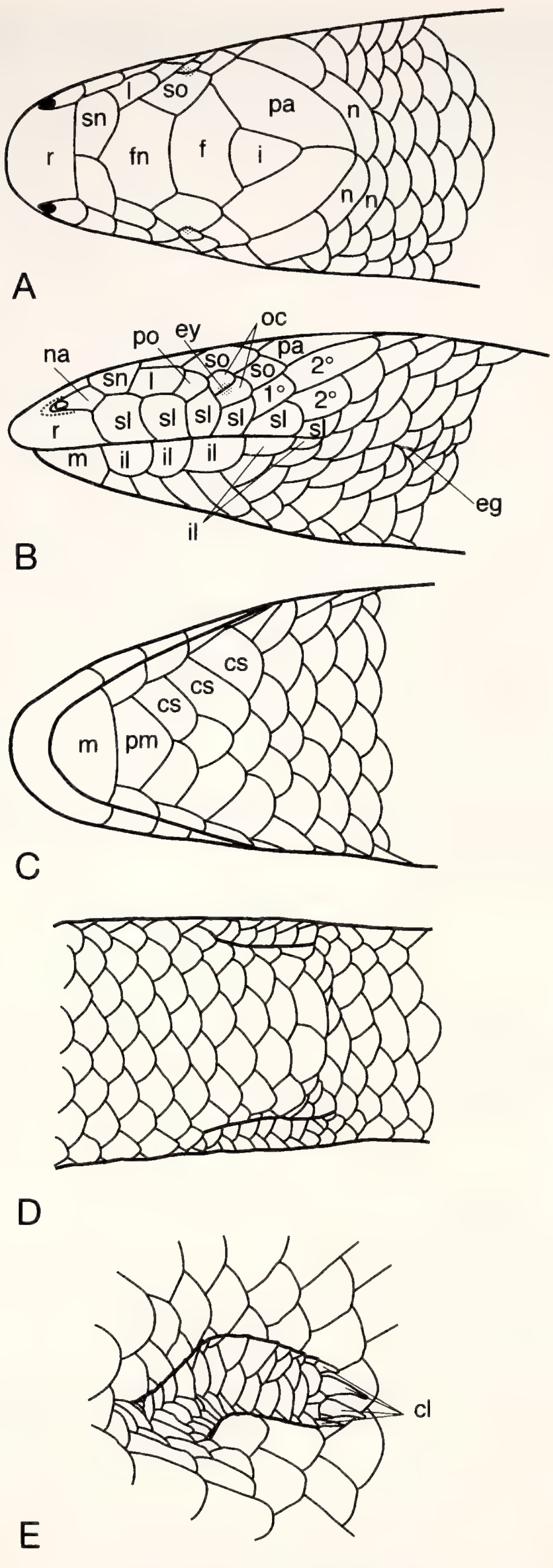

FIG. 3. Dorsal (A), lateral (B) and ventral (C) views of head, ventral view of cloacal region (D), and left forelimb (E) of the holotype of *Sirenoscincus yamagishii* sp. nov. (KUZ R50922). Abbreviations are: 1°, primary temporal; 2°, secondary temporal; cl, claw, cs, chinshield; eg, ear groove; ey, eye; f, frontal; fn, frontonasal; i, interparietal; il, inflalabial; l, loreal; m, mental; n, nuchal; na, nasal; pa, parietal; pm, postmental; po, preocular; oc, ocular; r, rostral; sl, supralabial; sn: supranasal; so, supraocular.

by one scale, and third separated by three scales; five infralabials, second highest, third widest; body and tail covered with smooth cycloid scales; position of hind limb insertion in other scincines having a patch of small scales, followed by short groove; preanal scales two, slightly larger than ventral body scales, right overlapping left; tail broken when captured.

Measurements of holotype (mm)

SVL, 82.5; original tail length, 84.4; head length, 7.1; snout length, 4.0; head width, 4.2; midbody width, 5.2; length of forelimb exclusive of claws, 3.8; fourth finger length, 0.6; distance between nostrils, 1.8.

Coloration

In life, head, body, forelimbs and tail uniformly pinkish white; snout somewhat paler than the other portion of head; black pigmentation of eye visible; autotomized tail somewhat whiter than original portion; scales transparent, without pigmentation; claws white. After preservation, pinkish coloration faded to uniformly white, and then to slightly yellowish after half year in alcohol.

Variation

In paratype, head slightly depressed; forelimb broken; SVL, 86.9; tail length, 53.7; head length, 6.20; snout length, 3.0; head width, 5.4; midbody width, 4.6; distance between nostrils, 1.7. This specimen had more nuchals than holotype—two in left, three in right.

Etymology

The generic name is derived from the Latin words, *siren* (mermaid) and *scincus* (skink), referring to the unique body shape of the type species with forelimbs only. The specific epithet is dedicated to Dr. Satoshi Yamagishi, who was a professor of Kyoto University and the project leader of Ecological Surveys in Ampijoroa, Ankarafantsika Strict Nature Reserve, in which both of the type specimens were obtained.

Natural history

The holotype was found under the leaf litter during a night survey. The collectors first found an autotomized tail which was still moving on the leaf litter. Then they searched around there and captured the holotype. The paratype was collected as a dead body on the road. Additionally three tails obviously belonging to the present species ware obtained as stomach contents of two colubrid snakes, *Liophidium torquatum* and *Dromicodryas bernieri*. These two snake species are considered to be terrestrial (Mori et al, unpublished observation). Probably *Sirenoscincus yamagishii* is a common prey item for such terrestrial snakes around the type locality.

Discussion

All Malagasy scincine lizards known to the present are fossorial or semifossorial exept for three aquatic or semi-aquatic species of the genus *Amphiglossus*; *A. astrolabi, A. reticulatus*, and *A. waterloti* (Brygoo, 1979, 1980a–d, 1981a–c, 1984a–d, 1985, Raxworthy and nussbaum, 1993), showing various degrees of reduction of limbs (from partial reduction of digits on forelimb or hind limb to the complete loss of both limbs), eyes, and ear-openings (Table 1). Among the eight genera, *Amphiglossus* seems to be most primitive in that it usually has four pentadactyl limbs, movable eyelids, and external ear openings. Three genera, *Voeltzkowia, Cryptoscincus*, and *Sirenoscincus*, are obviously extremely adapted to fossorial life in that their bodies are elongated, and eyes and ear openings are covered by scales. Pigmentation in scales is also lost in all the species of these genera exept for three species of *Voeltzkowia*.

Two species of *Voeltzkowia*, formerly assigned to the subgenus *Grandidiernina*, have no forelimbs and reduced hind limbs. The other three species of *Voeltzkowia* and a species of

TABLE 1. Comparisons of the Malagasy scincine genera. Numerals indicate numbers of digits on forelimbs and hind limbs. Symbols: +, present; –, absent; bt, button-like scale; nb, nub; st, styliform.

Genus/Species	Number of species	Eyes	Ear openings	Forelimbs	Hind limbs
Amphiglossus					
A. stylus	1	+	–	nb	st
A. crenni	1	+	+	2–3	2–4
other species	32	+	+	5	5
Androngo	1	+	+	2–5	2–5
Paracontias	8	+	–	–	–
Pseudoacontias					
P. angelorum	1	+	–	–	st
P. menamainty	1	+	–	bt	–
other species	2	+	–	–	–
Pygomeles					
P. braconnieri	1	+	+	–	st
P. petteri	1	+	–	–	–
Voeltzkowia					
V. fierinensis	1	–	–	–	2
V. petiti	1	–	–	–	st
other species	3	–	–	–	–
Cryptoscincus	1	–	–	–	–
Sirenoscincus	1	–	–	4	–

the monotypic genus *Cryptoscincus* (*C. minimus*) lack both forelimbs and hind limbs. In contrast, *Sirenoscincus* has tetradactyl forelimbs, but lacks hind limbs. This genus is quite similar to *Voeltzkowia* in a number of scale characters, but different from the latter in the combination of reduced limbs, suggesting its independent derivation from a four-limbed ancestor.

It is generally assumed that in Scincidae the limb loss occurred first in the forelimbs (Gans, 1975). *Sirenoscincus* offers a first obvious exception to these schemes. Another possible exception was exhibited by *Pseudoacontias menamainty*, which, while lacking hind limbs, has rudimentary, button-like traces of forelimbs. The bipedid amphisbaenians also have prominent forelimbs and lack hind limbs. From the shape of forelimbs that resemble those of moles, this group of lizards probably uses the forelimbs for digging. However the function of the forelimbs of *Sirenoscincus* is still unknown, since they seem to be too small for digging. The small but distinct forelimbs in *Sirenoscincus* may have a function for mating.

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Revalidation of *Rana laterimaculata* Barbour et Noble, 1916 from the Synonymy of *Rana baramica* Boettger, 1901

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Abstract: *Rana laterimaculata* Barbour et Noble, 1916 had been previously regarded as a junior synonym of *R. baramica* Boettger, 1901 by Inger (1966). An examination of literature, photographs, and live and preserved specimens revealed that visible and audible differences were apparent between two 'forms' of *R. 'baramica'*, which may even be sympatric in some localities. The acoustic characters of the two 'forms' are analysed and compared. *Rana laterimaculata* is here recognized as a valid species and a neotype is assigned in place of the missing holotype. The species is redescribed and diagnostic differences separating it from *R. baramica* are explained. The present known distribution of both species is provided. Both species are confined to the Sunda region.

Key words: Sunda; *Rana baramica*; *Rana laterimaculata*; Synonymy; Revalidation; Acoustics

Introduction

Rana baramica was first described by O. Boettger (1901) from Baram River, Sarawak, Borneo. A small series of specimens (syntypes: one male, two females, one sub-adult and four juveniles, collected by W. Kükenthal in 1894) were deposited at the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Germany (SMF), with detailed measurements provided for the three adult specimens (Table 1). Boettger specified diagnostic characters of very reduced toe webbing, a granular dorsum, lack of glandular folds, and distinctly widened toe tips, and mentioned that it was comparable with *R. signata* Günther. As no specific holotype was assigned by Boettger, a lectotype (SMF 4331, an adult female) was subsequently designated by Robert Mertens (1967).

Rana laterimaculata was described by Thomas Barbour and Gladwyn Kingsley Noble (1916) from Sadong, Sarawak, Borneo. The description was based on a single type speci-

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TABLE 1. Morphometric measurements (in mm) of three of the adult syntypes (one male, two females) of *Rana baramica* (after Boettger, 1901), alongside our own measurements of both females. SMF 4331 and 4332 correspond to Females #1 and #2 respectively.

	Three adult syntypes (After Boettger, 1901)			Examined by authors	
Measurements	Male	Female #1	Female #2	SMF 4331 (Lectotype)	SMF 4332
SVL	40	54	55	54.1	54.8
Head length	13½	18	18	18.3	18.7
Head width	12	17	16½	16.8	17.6
Tympanum	3½	4½	4½	3.9	4.5
Forelimb	27	35½	36	32.3	33.5
Hindlimb	68	87½	90	83.8	84.5
Tibia length	21½	28	28	28.9	28.6
Finger disc width	⅞	1	1	1.1	0.9
Toe disc width	1	1¼	1¼	0.8	1.0

men (sex and measurements not provided) collected by Harrison W. Smith in 1912 and deposited at the Museum of Comparative Zoology, Harvard, USA (MCZ 3811). Although Barbour and Noble did not specify any diagnostic details or make comparisons with other species, two characters were indicated (black tympanum and continuous white line on posterior half of upper lip) which may be used to separate *R. laterimaculata* from *R. baramica*. Up until 1932, both names were still regarded as valid, at least by Van Kampen (1923), although Boulenger (1920) did not list this species in his *Rana* monograph. However, *R. laterimaculata* was considered to be synonymous with *R. baramica*, according to Inger (1966), after examination of the holotype. Thereafter, frogs identified as belonging to this species have been recorded from other parts of Borneo: Sabah, Brunei, Kalimantan (Inger and Stuebing, 1997), Java, Sumatra, Bangka (Iskandar, 1998), Peninsular Malaysia (Berry, 1975; Dring, 1979; Manthey and Grossmann, 1997; Chan-ard et al., 1999) and Singapore (Lim and Lim, 1992).

The acoustic signal plays a very important role in preventing heterospecific breeding of anuran species, and is thus regarded as one of the key characteristics for clarifying taxonomic relationships of these animals (e.g., Matsui, 1997). In our observations and collections in the field, we found that there are two discrete call types in *R. baramica*. Subsequent studies involving literature reviews and examination of museum specimens revealed that *R. laterimaculata* deserves to be resurrected from synonymy with *R. baramica*. At least seven characters (six morphological, one behavioural - acoustic) may be used to distinguish between the two species, despite their superficial similarities. Additionally, in the course of this investigation, it was discovered that the holotype of *R. laterimaculata* is missing (José Rosado - MCZ herpetology curator, pers. comm.). Hence, another specimen (from type locality, previously identified as *R. baramica*) is designated as the neotype. *Rana laterimaculata* is redescribed in greater detail (based on specimens from Borneo, Singapore, Peninsular Malaysia and south Thailand) and comparisons are made with *R. baramica*.

MATERIALS AND METHODS

Field observations were conducted in Singapore, Malaysia (Peninsula: Johor, Pahang, Selangor; Sarawak: Mulu, Niah, Sibu, Sematan), and Indonesia (East Kalimantan: Bukit Soeharto, near Samarinda; Natuna Besar Island). Calls were mainly recorded at night in

the field using a cassette tape recorder (Sony TC-D5) with an external microphone (Sony ECM-23). Calls were later analysed from three to five individual males for each locality using the computer programs, SoundEdit Vers. 2 and SoundEdit Pro (MacroMind-Paracomp, Inc.) on a Macintosh computer. Terminology for acoustic characteristics follows Matsui (1997).

The types (lectotype and paralectotypes) of *R. baramica* were examined on loan from SMF, Germany. Specimens labeled as *R. 'baramica'* were examined from the collections of MCZ, FMNH (Field Museum of Natural History, Chicago), MZB (Museum Zoologicum Bogoriense, Java), NSM (National Science Museum, Thailand), DWNP (Department of Wildlife and National Parks, Peninsular Malaysia), ZRC (Zoological Reference Collection, Singapore), and KUHE (Graduate School of Human and Environmental Studies, Kyoto University, Kyoto). All measurements were made with slide calipers (to 0.1 mm). Denotions to specific digits are used as follows: for example, F1 and T1 for the first finger and toe, respectively. Degree of webbing between toes was determined by carefully stretching adjacent toes in order to examine its true extent.

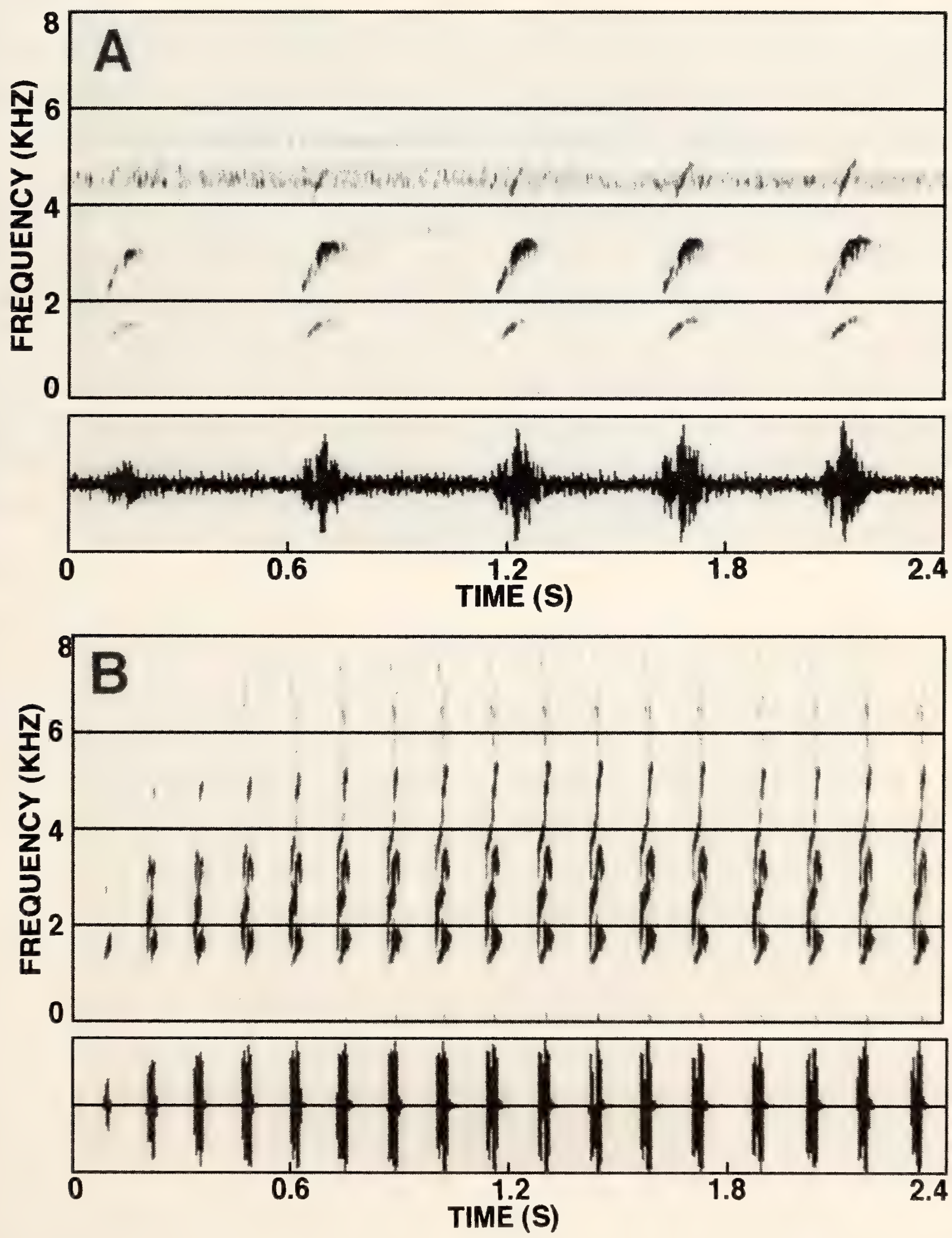

FIG. 1. Sonagrams (top) and wave forms (bottom) of advertisement calls of *Rana laterimaculata* Barbour et Noble from Sematan, Sarawak (A) and *R. baramica* Boettger from Niah, Sarawak (B).

Results

Acoustic Comparisons

Rana laterimaculata

We recorded the calls of frogs identified as *R. laterimaculata* at Sematan, Sarawak, on 5 September 1993 (air temperature 24.6 C), at Kuala Lumpur, Peninsular Malaysia, on 30 December 1992 (air temperature 26.4 C), and at Tempeler Park, near Kuala Lumpur, Peninsular Malaysia, on 21 January 1993 (air temperature 25.9 C).

This species has two call types. The normal advertisement call consists of a series of about 6–17 unpulsed notes and lasts about 1.9–7.5 s (Fig. 1A). The note repetition rate is 1.62–2.57 notes per second. Each note lasts 79–167 ms, and time interval between two notes varies from 333–800 ms. The dominant frequency lies at approximately 3000–3500 Hz, and harmonics are at about 1100–1700 and 4400–5000 Hz. The call has marked frequency and intensity modulations, and frequencies tend to increase once and then slightly decrease towards the end of a note. When calling actively, the normal advertisement call is often followed by several long notes and by a call of another type, which is slower and more intensive.

Rana baramica

The calls of 'true' *R. baramica* were recorded at Headquarters of Gunung Mulu, Sarawak, on 14 December 1989 (air temperature 24.3 C), at Niah, Sarawak, on 26 December 1990 (air temperature 26.1 C), at Sibu, Sarawak, on 17 January 1990 (air temperature 24.4 C), and at Bukit Soeharto, near Samarinda, East Kalimantan, on 1 August 1994 (air temperature 27.5 C).

This species also emitts calls of two types. The normal advertisement call (Fig. 1B) consists of a series of 14–30 unpulsed or weakly pulsed notes and lasts 2.0–6.3 s. The note repetition rate is 4.09–6.92 notes per second. Each note lasts about 12–81 ms, and time interval between two notes varies from 128 to 275 ms. The dominant frequency lies at 2000–2800 Hz, and harmonics are at about 1100–1900, 3200–3900, 5200–5400, and 7000–7200 Hz. The call has marked frequency and intensity modulations, and frequencies tend to increase towards the end of a note. Males often emit calls of another type during active calling just like *R. laterimaculata*. Calls of males from East Kalimatan differ slightly from those from Malaysia in that the main part of a call includes alternative successions of intensive and weak notes.

TABLE 2. Comparisons of normal calls between *Rana laterimaculata* Barbour et Noble and *R. baramica* Boettger.

Locality	AT (C)	n	Call L (ms)	N (notes)	Note L. (ms)	Note gap (ms)	Fund.Freq (kHZ)	Domin.Freq. (kHz)
R. laterimaculata								
Sematan	24.6	3	6785	14	138	511	1538	3000
Kuala Lumpur	26.4	4	4717	10	119	508	1515	3344
Tempeler	25.9	3	3330	8.3	117	430	1717	3388
x̄			4943.8	10.8	124.4	483.0	1590.0	3244.9
SD			1738.4	3.0	11.4	45.8	110.2	212.4
R. baramica								
Mulu	24.3	3	3741	22	34	173	1578	2009
Sibu	24.4	5	2449	16.8	44	151	1812	2344
Samarinda	27.5	4	4406	23.8	54	173	1774	2513
x̄			3532.0	20.9	44.0	165.7	1721.3	2288.7
SD			995.1	3.6	10.0	12.7	125.5	256.5

Thus, the normal advertisement calls are somewhat similar in the two species in that notes include marked frequency and intensity modulations, and that the normal advertisement call is followed by the call of another type. However, they clearly differ from each other in important characteristics (Fig. 1, Table 2) and are easily differentiated by the human ear. The number of notes in a call (mean=20.9 in *baramica* vs. 10.8 in *laterimaculata*) and call length (mean=3.5 s in *baramica* vs. 4.9 s in *laterimaculata*) slightly overlap between the two species, but the note and gap between notes are decidely shorter in *baramica* (mean=44.0 ms and 165.7 ms, respectively) than in *laterimaculata* (mean =124.4 ms and 483.0 ms, respectively), and the dominant frequency is lower in *baramica* (mean=2289 Hz) than in *laterimaculata* (mean=3245 Hz).

Redescription of Rana laterimaculata *Barbour et Noble*

Neotype

MCZ A-3885 [adult female, SVL (snout–vent length) 41.0 mm], Sadong, Sarawak, Borneo (type locality); collected by H. W. Smith (collector of holotype), date unknown (possibly around 1912). Additional measurements of neotype: head length 14.7 mm, head width 13.0 mm, body width 10.8 mm, axilla–groin distance 19.0 mm, interorbital distance 4.0 mm, internarial distance 3.4 mm, tympanum diameter 3.8 mm, tibia length 21.6 mm, foot length 18.7 mm.

Diagnosis

A small species of *Rana*, males (with humeral glands, nuptial pads, and paired subgular vocal sacs) to 39.0 mm, females (with unpigmented ova) to 47.5 mm; without dorsolateral skin folds; scattered tubercles over dorsum and flanks; finger and toe tips slightly expanded into discs not wider than twice the width of penultimate phalanx; webbing of fourth toe not reaching middle sub-articular tubercle on both sides; tympanum entirely black (dark brown in faded specimens); an uninterrupted white line on upper lip from posterior margin of tympanum to below eye. In life, actively calling males may be recognised by their high pitched "yip-yip-yip" calls.

Description

Habitus slender, head obtusely pointed, snout slightly rounded and projecting in profile; canthus rostralis distinct, lores concave; nostrils nearer to snout tip than to eye; tympanum conspicuous, ca. 3/4 eye diameter, with distinct supratympanic fold from behind eyelid to jaw angle; vomerine teeth in oblique rows, in line with and bounded by choanae.

Finger tips slightly widened, width not more than twice penultimate phalanx, with circum-marginal grooves; fingers long, F1 longer than F2, penultimate phalanx of F1 reaches tip of F2 when adpressed, F3 longest, F2 and F4 subequal; three metacarpal tubercles present, innermost one largest, middle and outer ones subequal; toe tips as in finger tips, slightly widened and bearing circum-marginal grooves; webbing between T1 and T2 reaching subarticular tubercle of T1 but not that of T2, webbing between T2 and T3 just surpassing subarticular tubercle of T2 and reaching proximal subarticular tubercle of T3, webbing between T3 and T4 reaching distal subarticular tubercle of T3 and just surpassing proximal subarticular tubercle of T4, webbing between T4 and T5 surpassing proximal subarticular tubercle of T4 and just surpassing distal subarticular tubercle of T5; an elongated oval inner metatarsal tubercle and a rounded outer metatarsal tubercle; fingers and toes without peripheral dermal fringes or flaps.

Dorsum and flanks with distinctly raised granules and tubercles, some forming short longitudinal ridges along flanks; tibia with parallel longitudinal dermal ridges; venters smooth, rugose on undersides of femur and around vent.

In life, basal colour of dorsum and flanks brown, with darker brown to black blotches and spots (more conspicuous on flanks); tubercles along dorsolateral region usually of lighter

shade; limbs also brown with darker brown barring/blotches extending onto fingers and toes; tympanum entirely black; an uninterrupted white streak from posterior margin of tympanum to just below eye; iris yellowish in dorsal third, reddish brown in ventral two thirds; throat and chest heavily mottled with dark brown, often with a white dividing line from chin towards sternum; belly and underside of femur with reduced pigmentation. In preservative, a slight fading of the original colour occurs.

Sexual dimorphism

Males a little smaller than females, SVL of males to 39.0 mm, females to 47.5 mm. Males possess distinctly bulbous humeral glands, nearer to axilla than elbow; paired subgular vocal sacs; finely granular, unpigmented nuptial pads on first finger arranged in two separate groups, one opposite the subarticular tubercle and the other immediately adjacent to the inner metacarpal tubercle. Females with unpigmented ova. A gravid female from Singapore (ZRC. 1.4763) aborted her ova (ZRC.1.4764, ca. 400 counted) in captivity and the ova had a diameter of 1.3–1.6 mm.

Natural history

Predominantly inhabitant of lowland freshwater and peat swamp forests. Males call from among or atop leaf litter of forest floor, or perched on low vegetation up to one metre above ground. Call of male consists of an initially ascending series of high-pitched "yip-yip-yip" accelerating towards the end. *Rana laterimaculata* is syntopic with *R. baramica* in some parts of its range, and may be found with other Sundaic lowland swamp forest anurans, such as *Bufo quadriporcatus, Leptobrachium nigrops, Limnonectes paramacrodon, Phrynoglossus laevis, Rana glandulosa, Microhyla borneensis*, or *Rhacophorus appendiculatus*. Neither in situ nor ex situ amplexus has been observed. Its diagnostic larval identity remains as yet unknown.

Geographic distribution

Apart from the type locality, Sadong, Sarawak, and newly recorded locality, Sematan, Sarawak, other Bornean records have not been reported. Healthy populations occur within the inland swamp forests of Singapore (Central Catchment Nature Reserve, Bukit Timah Nature Reserve) and Peninsular Malaysia (Johor, Selangor, Pahang, Trengganu). It has been collected from similar habitats as far north in the peninsula as south Thailand (Hala Bala Wildlife Sanctuary - NSM voucher specimens). Its distinctive calls were heard in peat swamp forest on Natuna Besar Island (South China Sea), Indonesia. The populations from Singapore, Johor, and Natuna Besar Island are syntopic with *R. baramica* (T. M. Leong, pers. obs.).

DISCUSSION

In the field, calling males of *R. laterimaculata* may be detected by their distinctive high pitched "yip-yip-yip", as previously recorded and analysed by Julian C. M. Dring (1979). *R. baramica* instead calls with a rapid series of "kwuck-kwuck-kwuck", as observed in Peninsular Malaysia, Singapore, Borneo and Natuna Besar. Once located, frogs may be positively identified as *R. laterimaculata* (Fig. 2) by

FIG. 2. Adult male *Rana laterimaculata* Barbour et Noble from Singapore. Note black tympanum and continuous white streak (along upper lip) from beneath eye to posterior margin of tympanum.

noting the following combination of characters: (a) tympanum entirely black, without gold spot in center, (b) upper lip with a continuous white streak from beneath eye to posterior margin of tympanum, (c) iris yellowish in dorsal third, reddish brown in lower two thirds. While the first two characters are retained upon preservation, the iris colour is not. Further examination of preserved specimens, where available, will confirm its identity as *R. laterimaculata* by checking that: (a) webbing between T4 and T5 does not reach middle subarticular tubercle of T4, (b) SVL of males

FIG. 3. Adult male *Rana baramica* Boettger from Singapore. Note gold spot in centre of brown tympanum and interrupted light and brown barrings along upper lip.

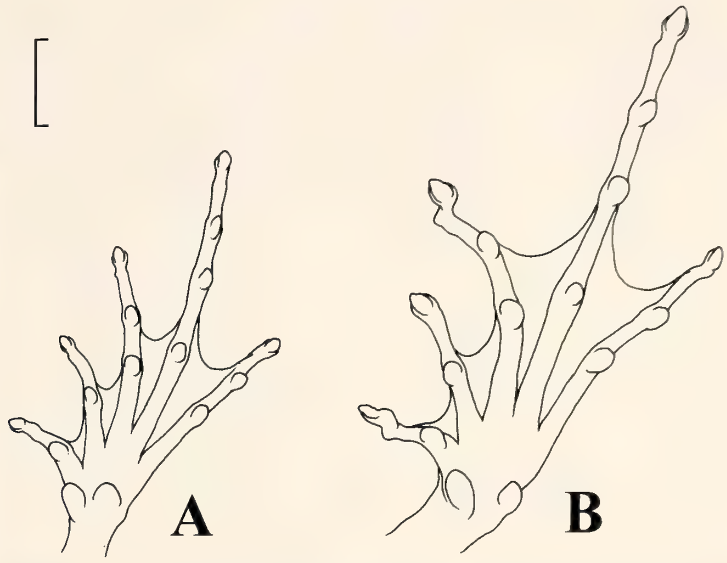

FIG. 4. Ventral aspects of the left hindfeet of (A) *Rana laterimaculata* and (B) *R. baramica*, illustrating their relative sizes and degree of webbing (note fourth toe). Both illustrated from adult male specimens (ZRC.1.10033 and ZRC.1.6111 respectively) collected in Singapore. Scale bar equals 3 mm.

FIG. 5. Preserved specimens of adult male (left) and female (right) *Rana laterimaculata* Barbour et Noble (above) and *R. baramica* Boettger (below) from Singapore, illustrating the size differences between sexes of both species.

not greater than 39.0 mm, females not exceeding 47.5 mm, (c) pineal spot not visible.

Rana baramica (Fig. 3), on the other hand, may be distinguished from *R. laterimaculata* by the following characters: (a) tympanum brown with distinct gold spot in center, (b) upper lip with alternate light and dark bands throughout, (c) webbing between T4 and T5 just reaching middle subarticular tubercle of T4, (d) SVL of males to 55.6 mm, females to 70.9 mm, (e) presence of light gold pineal spot. In life, its iris is golden in dorsal and ventral halves, with a tinge of red at the lateral corners. The differences between both species are summarised in Table 3. Comparisons between the relative size/webbing of their hindfeet are illustrated (Fig. 4). The size differences between adults of the two species are significant, with males and females of *R. baramica* up to 40% and 50%, respectively, larger than those of *R. laterimaculata* (Fig. 5).

True *Rana laterimaculata* occurs throughout Borneo (Sabah, Sarawak, Brunei, Kalimantan), Java, Sumatra, Bangka, Natuna Besar, Peninsular Malaysia (Johor, Selangor) and Singapore. Previous references using the name of *R. baramica* for Peninsular Malaysia (Berry, 1975; Dring, 1979 Manthey and Grossmann, 1997; Chan-ard et al., 1999) and Singapore (Lim and Lim, 1992) actually refer to true *R. laterimaculata* instead. For Bornean *R. baramica*, the photograph in Inger and Stuebing (1997: Fig. 66, p. 128) depicts a recent metamorph, lacking the essential diagnostic characters of the adult. However, a more accurate specimen (male) of this species is available in Inger and Tan (1996: Fig. 41, pg. 29). As mentioned above, both *R. laterimaculata* and *R. baramica* are known to be syntopic in Singapore, Johor (southern Peninsular Malaysia), and Pulau Natuna Besar (South China Sea). As far as we could discern from presently available specimens, there were no great variations between populations of either *R. laterimaculata* or *R. baramica* from various localities (e.g., beteen Borneo and Peninsular Malaysia) that might have suggested an extended period of geographic isolation from each other.

Another Malaysian species, *R. glandulosa*, shares a common character with *R. laterimaculata* and *R. baramica* in that its ova are unpigmented. The species, however, may be

TABLE 3. Comparison of characters differentiating *Rana laterimaculata* Barbour et Noble from *R. baramica* Boettger. (T4 and T5 denote fourth and fifth toes, respectively)

Character	*R. laterimaculata*	*R. baramica*
Tympanum	Entirely black	Light to dark brown, with distinct gold spot in the centre
Upper lip	Continuous white streak from beneath eye to posterior margin of tympanum	Similar light streak but interrupted in between by brown bands
Hind foot	Webbing between T4 and T5 not reaching middle subarticular tubercle of T4	Webbing between T4 and T5 just reaching middle subarticular tubercle of T4
Size range (SVL)	Males: 28.3–39.0mm Females: 39.6–47.5mm	Males: 36.5–55.6mm Females: 49.5–70.9mm
Pineal spot	Not visible	Distinct light gold spot at anterior margin of interorbital area
Iris colour (live)	Yellowish in dorsal third, reddish brown in lower two thirds	Golden in dorsal and ventral halves with tinge of red at the laterals
Vocalisation (perception by ear)	High pitched “yip-yip-yip···”	Rapid “kwuck-kwuck- kwuck···”

clearly distinguished from either *R. baramica* or *R. laterimaculata* by its loud, slow series of "whack-whack-whack" calls. It is also a much larger *Rana*, with SVL of males to 93 mm and females to 84 mm (males larger than females, instead of vice versa); it has more rounded, pustulose glandules instead of tubercles on its dorsum and sides; its toe webbing is also slightly more extensive (Inger, 1966; Inger and Stuebing, 1997). Despite local abundance of these three species in certain preferred habitats, neither mating behaviour nor diagnostic larvae have been reported thus far.

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APPENDIX

Material Examined

Rana baramica

Java: MZB 2927 (Batu Gede, Ciberut, north Bogor); Sumatra: MZB 2925 (Mapala nabita, Riau), MZB 3932 (Musibanyuasin, Hulu Sungai Pejudian, South Sumatra); Borneo: SMF 4331 [lectotype], 4332–4335 [paralectotypes] (Baram River, Sarawak); FMNH 249868–249875 (Beaufort District, Sabah), FMNH 119721–119725, 131982–131985, 146436–146553, 148006–148047 (4th Division, Bintulu District, Sarawak), MZB 883 (Mentawir, East Kalimantan), MZB 2579 (Pontianak, West Kalimantan), MZB 2859 (Taman Nasional Gunong Palung, West Kalimantan), ZRC.1.4638–4639 (Long Iram, East Kalimantan), ZRC.1.7690 (Bentong, Sarawak), ZRC.1.9017 (Niah, Sarawak), ZRC.1.9456 (Kota Samarahan, Sarawak), ZRC.1.9460–9462 (Balai Ringin, Sarawak), ZRC.1.9486 (Bako, Sarawak), MCZ A-64832 (Nyabu Forest Reserve, Bintulu, Sarawak), KUHE 12079, 12082–12085, 12116–12121 (Niah, Sarawak), KUHE 10537, 17017, 17030–17031 (Mulu, Sarawak), KUHE 17068, 17119 (Gading, Sarawak); Peninsular Malaysia: ZRC.1.3027 (north Selangor peat swamp forest), 8032, 10386 (Panti, Johor); Singapore: ZRC.1.1400–1402, 4761 (Nee Soon swamp forest), ZRC.1.3491, 5199, 6107–6111, 6291–6293, 9178, 10034–10035, 10402–10403 (Sime road swamp forest).

Rana laterimaculata

Thailand: NSM C-154, 3869, 3877–3881, 3980 (Hala-Bala Wildlife Sanctuary, Narathiwat Province); Peninsular Malaysia: FMNH 186156–186266, 191491 (Bukit Lanjan, Selangor), DWNP.A.0285, 0677 (FRIM, Kepong, Selangor), DWNP.A.0286–0287 (Sungkai, Perak), DWNP.A.0288 (Sungai Teris, Pahang), DWNP.A.1200 (Gunong Belumut, Johor), ZRC.1.303 (Baling, Kedah), ZRC.1.1114–1115 (Bukit Chintamani, Pahang), ZRC.1.5490, 5555 (FRIM, Kepong, Selangor), ZRC.1.10056 (Sungai Seneku, Lakun Forest Reserve, Bentong, Pahang), ZRC.1.10157 (Raub, Pahang), ZRC.1.10387 (Panti, Johor), ZRC.1.10748 (Bekok, Johor), KUHE 15677–15678 (Templer, Selangor); Singapore: ZRC.1.1540, 5603, 6116, 9179–9180, 9656–9658, 10032–10033, 10220–10223, 10404–10405 (Sime road swamp forest), ZRC.1.2929, 3016, 4762–4763 (Nee Soon swamp forest), ZRC.1.5279 (Rifle Range road swamp forest), ZRC.1.10279–10281, 10408, 10524 (Lower Peirce boardwalk, swampy area beside stream); Borneo: MCZ A-3885 (Sadong, Sarawak) [neotype], KUHE 17592–17594, 17607, 17650 (Sematan, Sarawak).

Rana glandulosa

Sumatra: MZB 918 (Sungai Siak, Central Sumatra), MZB 3263 (Rantau Prapat, Labuhan Batu, North Sumatra), MZB 6902 (Sungai Pasilog, Bukit Lawang, North Sumatra), MZB 3644 (Padang, West Sumatra), MZB 3755–3759 (Pulau Pini, north of Siberut), MZB 3322 (Tasik Serai, Mandau, Riau), MZB 893–895 (Sungai Tanajan, Pekan Baru, Riau); Thailand: CUHC 1072–1074 (Bhetong, Yala Province), NSM 4354 (Hala, Yala Province); Peninsular Malaysia: DWNP.A.0427 (Tasek Bera, Pahang), DWNP.A.0428 (Paya Indah, Kuala Langat, Selangor), DWNP.A.0429, 0430, 0433, 0434 (Krau Wildlife Reserve, Pahang), DWNP.A.0431–0432 (Sungkai Forest Reserve, Perak), DWNP.A.0566 (Kual Koh, Kelantan), DWNP.A.1133–1135 (Sungai Ambat, Tenggaroh, Mersing, Johor), DWNP.A. 1166–1168, 1171 (Lubuk Rincing, Sungai

Keniam, Taman Negara, Pahang), ZRC.1. 3881 (Batu Pahat, Johor), ZRC.1.6071 (Sungai Wang Burma forest, Perlis), ZRC.1.7956–7957 (Bukit Rengit, Pahang), ZRC.1.8614 (Kahang, Johor), ZRC.1.9164 (Sungai Kancing, Selangor), ZRC.1.10053 (Sungai Seneku, Lakun Forest Reserve, Bentong, Pahang), ZRC.1.10149–10150 (Raub, Pahang); Borneo: MZB 3806 (Sungai Bulit, Karimun, West Kalimantan), MZB 2564–2565 (Kapuas, West Kalimantan), FMNH 146460 (Nyabau, Sarawak).

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A New Water Skink of the Genus *Tropidophorus* (Lacertilia: Scincidae) from Sulawesi, Indonesia

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Abstract: The population of the lygosomine genus *Tropidophorus* on Sulawesi, Indonesia, is usually referred to as *T. grayi* Günther, 1861, a species originally described from the Philippines, or sometimes by a taxonomically unjustified epithet "*apulus*". We therefore reexamined the taxonomic status of the Sulawesi *Tropidophorus* on the basis of newly obtained specimens and available museum materials. Results indicated that this island population is morphologically distinct from *T. grayi*, and we thus describe it as a new species. This species most resembles *T. grayi* by sharing a number of characteristics, such as a divided frontonasal, three preanals, and strongly keeled tail scales. However, it differs from the latter by having weaker keels on dorsal body scales, a relatively longer fourth toe with more subdigitals, and male postanal pores in a single row.

Key words: *Tropidophorus grayi*; New species; Scincidae; Sulawesi; Indonesia

Introduction

When two of us (TH and HO) in 1999 visited the Herpetology Section of the Natural History Museum, London, we found one scincid specimen labelled as "holotype of *Tropidophorus aphilus*". This specimen (BMNH 1926.10.30.71) was catalogued as being collected from Celebes (=Sulawesi) by Malcom A. Smith.

Through a personal communication with Dr. Robert F. Inger at Field Museum of Natural History, we learned that his former student, Dr. James P. Bacon, had been preparing a manuscript to describe the Sulawesi *Tropidophorus*, usually referred to as *T. grayi* Günther, 1861, then (see below), as a new species with holotype designation of that BMNH specimen. Unfortunately Dr. Bacon's manuscript was not completed for publication because of his death in 1986.

Recently several pet dealers, in their advertisement price catalogues on the Internet and in some hobbyists' magazines, started to refer

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to the water skink from Sulawesi as *Tropidophorus apulus*, most likely by erroneously copying Bacon's unpublished name, *T. aphilus*. However, neither of these names are actually available in accordance with International Code of Zoological Nomenclature, because the name *Tropidophorus aphilus* remains unpublished (see above) and the name *Tropidophorus apulus*, when appearing in hobbyists' magazines, has never been accompanied by information to fill the minimum requirements of the code as an original description of a new species-group name, such as that regarding the holotype and diagnostic characters (International Commission on Zoological Nomenclature, 1999).

The genus *Tropidophorus* belongs to the subfamily Lygosominae of the family Scincidae, and is characterized by more or less aquatic habits and a few morphological features, such as a superficially located tympanum. A total of 22 species, distributed in both continental and insular parts of Southeast Asia, are currently recognized for this genus (Smith, 1923; Taylor, 1963; Brown and Alcala, 1980; Zhao and Adler, 1993; Hikida and Ota, 1994; Manthey and Grossmann, 1997; Hikida et al., 2002). In most scientific publications except Brown and Alcala (1980: see Discussion for details), the Sulawesi population of *Tropidophorus* has been referred to as *T. grayi* following Rooij (1915), who first examined the taxonomic status of this island population on the basis of specimens from "Luhu and Makassar (=Ujung Pandang), Celebes" (Fig. 1). *Tropidophorus grayi* was originally described from the Philippines (Günther, 1961), and has been recorded from Luzon, Polillo, Leyte, Negros, Mastabe, and Cebu of this archipelago (Brown and Alcala, 1980), besides Sulawesi.

Recently one of us (AR) collected two additional specimens of *Tropidophorus* from South Sulawesi. Comparisons of these and other specimens from Sulawesi with specimens of *T. grayi* from the Philippines confirmed their distinct morphological differences as had been implied by Bacon's uncompleted attempt

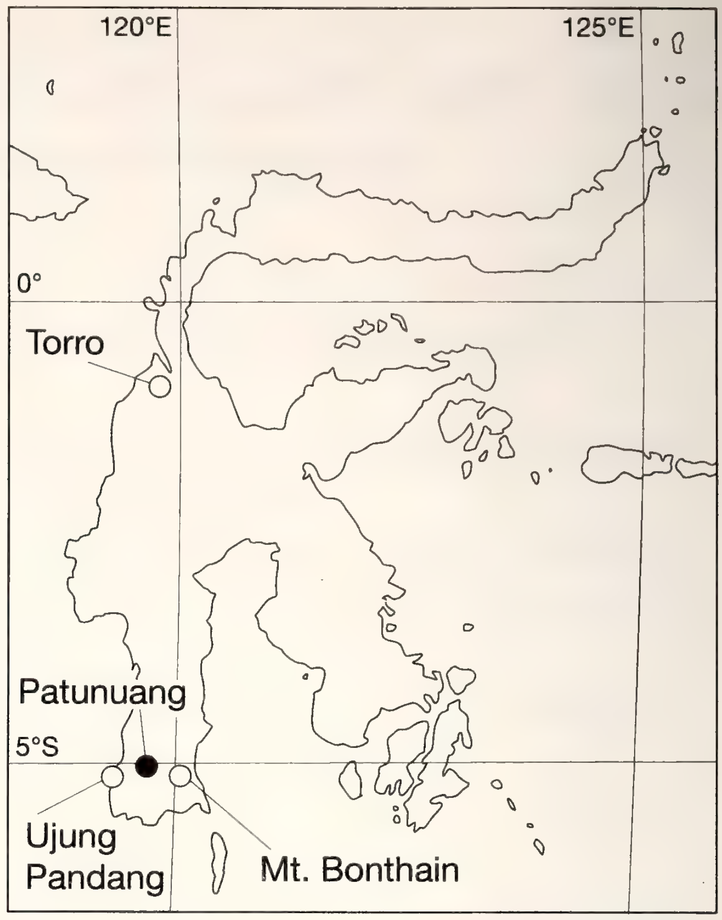

FIG. 1. Map of Sulawesi, showing sampling localities of *Tropidophorus baconi* sp. nov. Closed circle indicates the type locality.

(see above). We thus describe the Sulawesi population of *Tropidophorus* as a new species.

Materials and Methods

A total of six specimens of *Tropidophorus* from Sulawesi, Indonesia, were examined and compared with 22 *T. grayi* from the Philippines (including three syntypes of the species and the holotype of *Enoplosaurus insignis* Sauvage, 1879, a junior synonym of *T. grayi*) (see Appendix for further details of specimens used for comparisons). Comparisons with other congeneric species were made on the basis of specimens and literature referred to in our previous paper (Hikida et al., 2002).

Measurements were taken to the nearest 0.1 mm with dial calipers. Paravetebral scales were counted following Greer (1982). In a previous paper (Hikida et al., 2002), we proposed a new name, postsupraocular, to refer to a distinctly smaller scale behind the supraocular series, which had usually been

treated as the fifth supraocular (Rooij, 1915) or the last supraciliary (Taylor, 1936). However, this scale was actually already discriminated from supraoculars and supraciliaries under a different name, pretemporal, by Greer and Nussbaum (2000). In the description below, we, therefore, use this reference name instead of postsupraocular. For convenience for comparisons on the basis of previously published data, we follow Taylor's (1936) definitions for other scale characters because of their broad uses in subsequent works dealing with scincid systematics and taxonomy.

Catalogue numbers of specimens deposited in the Zoological Collection of the Kyoto University Museum are preceded by KUZ. Other institutional acronyms follow Leviton et al. (1985).

Tropidophorus baconi sp. nov.
Figs. 2 and 3

Holotype

MZB.Lace 3789 (Field No. AR 00053), subadult male, collected from Patunuang Natural Reserve (5°03'07"S, 119°43'07"E), South Sulawesi, Indonesia, by A. Riyanto on 31 May 2001.

Paratypes

MZB.Lace 3788 (Field No. AR 00166), adult female, with sampling data same as the holotype; BMNH 1926.10.30.71, adult female, collected from Lowah, Mt. Bonthain, Celebes, Dutch East Indies (=South Selawesi, Indonesia), by M. A. Smith; BMNH 96.12.9.42, juvenile male, collected from Luhu (exact location not determined), Celebes, by S. and F. Sarasin; BMNH 1980.905, juvenile of unknown sex, collected from Torro, Kabupaten Donggala, Central Sulawesi (alt. 600 m; 01°27'S, 119°59'E) (collector unknomwn). KUZ R38805, adult female, obtained from a pet dealer (exact locality unknown).

Diagnosis

A relatively large *Tropidophorus*, 87–120 mm in SVL. This species differs from

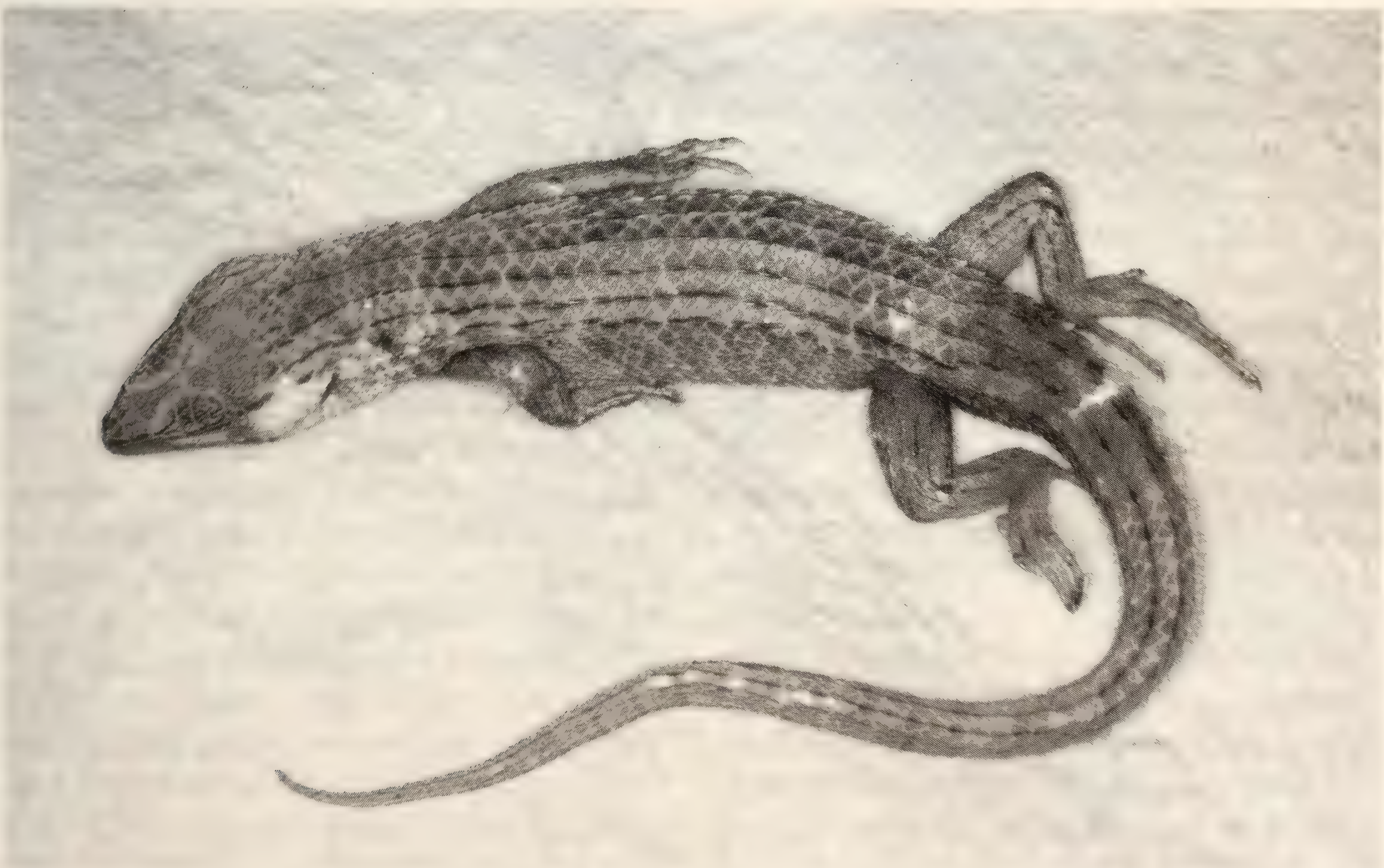

FIG. 2. Dorsal view of the holotype of *Tropidophorus baconi* sp. nov. (MZB.Lace 3789, SVL=87.0 mm).

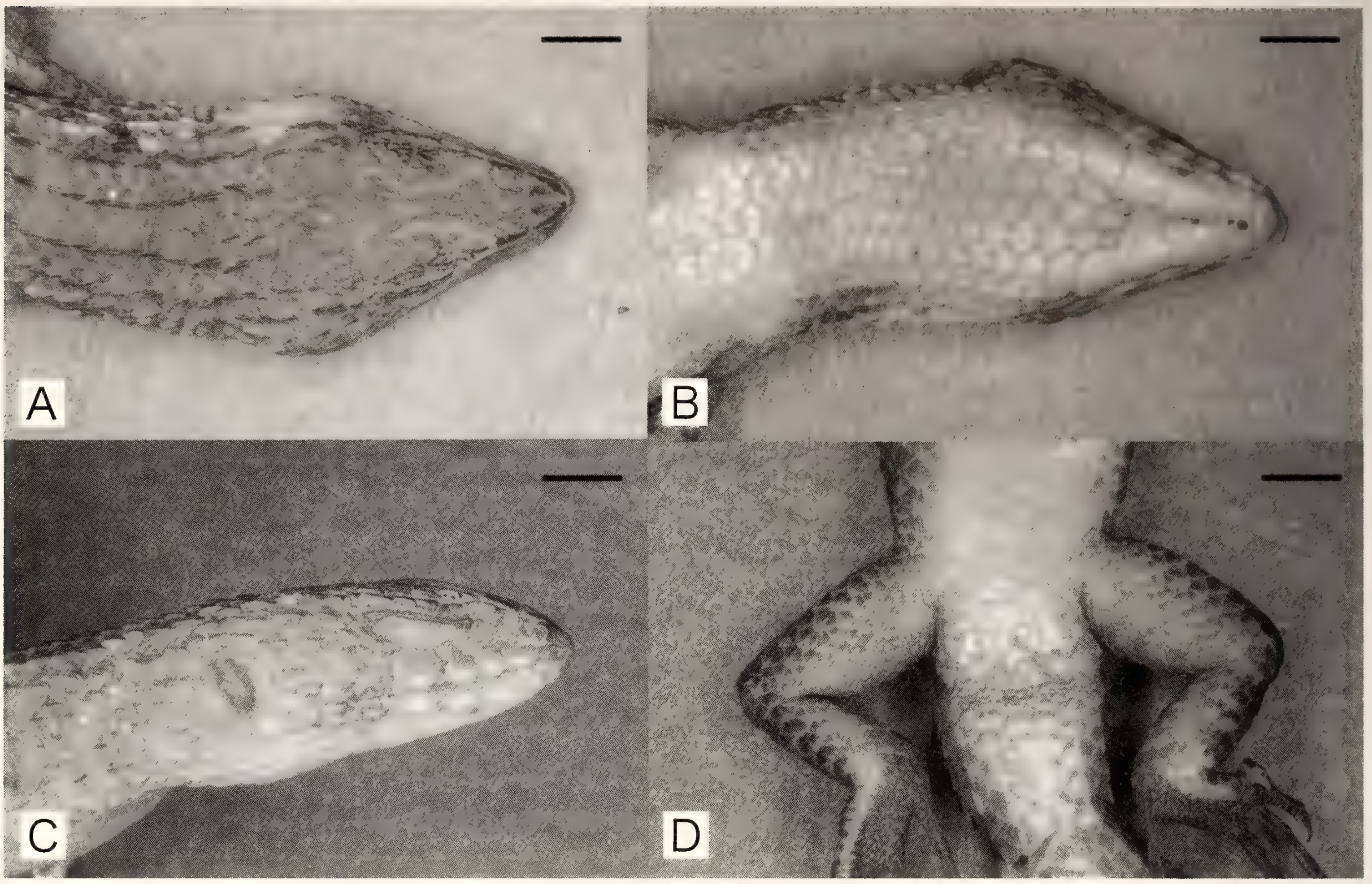

FIG. 3. Dorsal (A), ventral (B) and lateral (C) views of head, and ventral view of cloacal region (D) in the holotype of *Tropidophorus baconi* sp. nov. (MZB.Lace 3789). Bar equals 5 mm.

other congeneric species except *T. grayi* by having a divided frontonasal, parietals separated from each other, lateral body scales directed obliquely upward (Fig. 4), three preanals, postanal pores in adult males, and single keels on dorsal body and tail scales with marked development of the latter to make the tail surface more or less spinose. Besides the preanal scale number, most other scale counts also largely or completely overlap between the two species (Table 1). Nevertheless, *T. baconi* is distinct from *T. grayi* in having relatively longer toes and more subdigitals thereon (22–27 vs. 17–20) with a larger number of proximal ones medially divided (8–12 vs 2–3: Fig. 5). *Tropidophorus grayi* has strongly striated scales on dorsal and lateral surfaces of head, and distinctly keeled spinose scales also on dorsal surfaces of body, whereas in *T. baconi*, striations in head scales are much weaker and dorsal body scales are not spinose (Fig. 4). Postanal pores in males form a single row in *T. baconi*, but three rows in *T. grayi* (Table 1).

Description of holotype

A subadult male (Fig. 2), with undeveloped testes. Temporal region on left side of head partially injured.

Head scales rugose, weakly striated; snout rounded, rostral partially visible from above, overlapping frontonasals, nasals, and first supralabials; no supranasals; frontonasal divided, overlapped by rostral, nasals, and upper anterior loreals, overlapping prefrontals; left frontonasal widely overlapped by right one; prefrontal overlapped by frontonasal and loreals, overlapping frontal, first supraocular and first superciliary; left prefrontal widely overlapped by right one; frontal large, narrowing posteriorly, overlapped by prefrontals, overlapping first and second supraoculars and frontoparietals; supraoculars four, overlapped by superciliaries; first supraocular with distinct ridge; superciliaries six, first two large, remaining four distinctly smaller; interparietal narrowing posteriorly, overlapping parietals, with small transparent spot

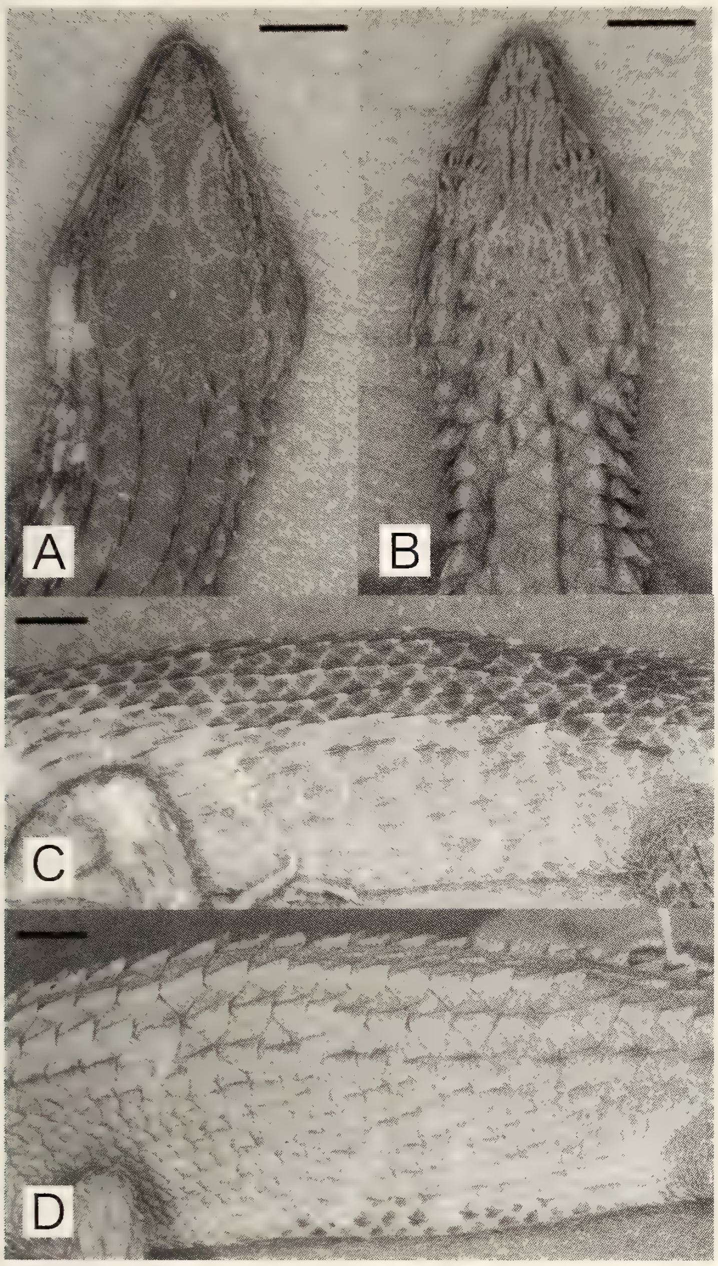

FIG. 4. Dorsal views of heads and dorsolateral views of bodies of *Tropidophorus baconi* sp. nov. (A and C, respectively: holotype, MZB.Lace 3789) and *T. grayi* from Luzon, Philippines (B and D, respectively). Bar equals 5 mm.

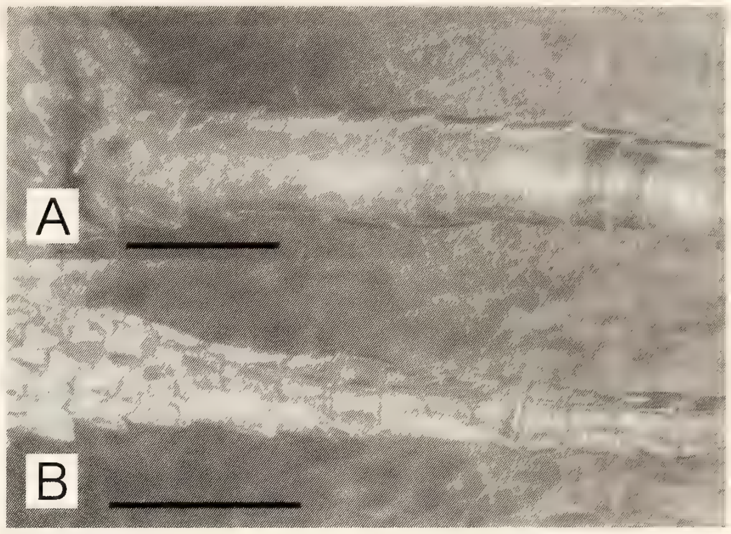

FIG. 5. Ventral views of fourth toe bases in a paratype of *Tropidophorus baconi* sp. nov. (A: MZB.Lace 3788) and *T. grayi* from Luzon, Philippines (B). Bars equal 2 mm.

showing location of parietal foramen; parietals separated by one scale; no distinct nuchals; nostril piercing nasal; nasal overlapped by rostral and first supralabial, overlapping frontonasal and loreal; anterior loreal overlapped by nasal, overlapping frontonasal, prefrontal, upper and lower posterior loreals, and second supralabial; posterior loreals divided into larger upper and smaller lower elements; supralabials seven, including four preorbital, one subocular, and two postocular ones; subocular supralabial bearing shallow groove continuing from one between preorbital supralabials and loreals; postocular supralabials keeled; presuboculars three, overlapped by posterior loreal and third and fourth supralabials; one tiny scale between first presubocular and third supralabial; lower eyelid with two rows of six or seven relatively large scales, separated from subocular supralabials by one row of granular scales; postoculars three, overlapped by fourth supraocular and palpebrals, overlapping postsuboculars; pretemporal keeled, overlapped by fourth supraocular and postocular, overlapping parietal and primary temporal; postsuboculars five, keeled, first largest, overlapped by fifth supralabial and overlapping sixth supralabial; temporals keeled in four rows, those in second and third rows enlarged; uppermost secondary and tertiary temporals overlapped by parietal; tympanum superficial; mental overlapping first infralabials and postmental; postmental undivided, overlapping first chin shields; chin shields in three pairs, first left overlapped by right, second pair separated by single scale, third pair separated by three scales; infralabials six, second–fourth larger than others; midbody scales in 26 rows; tail scales in 13 rows at position of tenth subcaudal; paravertebrals (mid-dosal scales from posterior end of parietals to position just above posterior margin of thigh) 34, as wide as neighboring scales; each of dorsal and lateral scales with single keel; scales on tail strongly keeled; dorsolateral scales on neck also

TABLE 1. Comparisons of morphological characters between *Tropidophorus baconi* sp. nov. and *T. grayi*.

Character	*T. baconi* sp. nov.	*T. grayi*
SVL	87.0–120.0	82.6–119.3*
head scales	rugose	strongly striated
dorsal scales	not spinose	spinose
midbody scale rows	24–27	24–28*
paravetebrals	29–34	30–34*
subdigitals of 4th toe	22–27 (8–12)**	16–21* (2–3)**
postanal pores	1 rows	3 rows

* Including data from Brown and Alcala (1980).
** Number of divided subdigitals at base in parentheses.

strongly keeled; lateral body scales obliquely directed; ventral scales in eight rows, weakly keeled; first subcaudal divided, remaining ones entire, keeled, gradually narrowing to become as wide as neighboring scales posteriorly; preanals three, enlarged, middle one overlapped by those on both sides; postanal pores 15, forming single row; scales on forelimb keeled, those on hind limb distinctly keeled dorsally, weakly keeled ventrally; 26–27 subdigitals beneath fourth toe, basal eight divided, remainder entire.

Measurements of holotype (mm)

Snout to vent length (SVL), 87.0; tail length, 121.0; axilla to groin length, 40.9; snout to forelimb length, 34.7; head length (tip of snout to posterior margin of parietals), 19.1; head width, 16.5; head depth, 10.7; eye length, 6.5; snout length (tip of snout to anterior corner of eye), 8.0; eye to ear-opening length, 8.2; snout to ear-opening length, 20.1; forelimb length, 31.5; hind limb length, 43.2; fourth toe length, 13.9; tympanum height, 4.5; tympanum width, 4.2.

Color in preservative

Dorsal and lateral ground color of head, body, and tail dark brown; three and six indistinct slightly lighter transverse bands on neck and dorsum of body, respectively; ventral surfaces of head and body yellowish white, slightly darker in gular region; ventral surface of tail light brown in anterior region, darker posteriorly.

Variation

Of paratypes, three adult females and two juveniles measured 89.1–120 and 36.6–47.7 mm in SVL, respectively. The number of paravertebrals varied from 29 to 33. The numbers of midbody scale rows and tail scale rows were 24–27 and 11–13, respectively. The numbers of all and divided subdigitals on fourth toe ranged from 22 to 27 and 9–12, respectively. Other scale counts were same as those in the holotype. Females had no postanal pores.

Etymology

The name is dedicated to the late Dr. James P. Bacon in recognition of his contributions to the taxonomy of the genus *Tropidophorus*.

Natural history

The holotype and one female paratype (MZB.Lace 3788) were found in a small stream with a rocky bottom. When located, the holotype was resting with its body half submerged in water near the grassy shore, and the female was sitting on a large rock in the stream.

Distribution

Central and South Selawesi, Indonesia (Fig. 1).

Remarks

Rooij (1915) first reported *T. grayi* from Sulawesi on the basis of specimens collected from Luhu and Makassar (Ujung Pandang). Later, Brown and Alcala (1980) did not include Sulawesi in the known range of distribution of the species, and regarded *T. grayi* as being endemic to the Philippines. It is thus highly likely that Brown and Alcala (1980) already supposed the Sulawesi *Tropidophorus* to be different from *T. grayi*, although they did not provide any reason for the above treatment. Probably, they knew of and followed Bacon's idea to recognize the Sulawesi population as a distinct species by itself (see Introduction above). Because their book (Brown and Alcala, 1980) accommodates Bacon's description of *T. davaoensis* from Mindanao, Philippines (Bacon, 1980), it is obvious that Brown and Alcala had close contact with him then.

Acknowledgments

TH and HO thank M. Matsui for providing opportunities to visit museums in Europe, and AR thanks Hary Wawangningrum for her assistance with his part of this study. We are also much indebted to E. N. Arnold and C. McCarthy (BMNH), I. Ineich and A. Dubois (MNHN), G. Köhler (SMF), Siti Nuramaliati Prijono and Mumpuni Sancoyo (MZB), and H. Endo (NSMT) for allowing us to examine pertinent specimens in their care. Surveys on the European museum collections were financially supported by a Monbusho International Scientific Program, Field Research No. 10041166, (project leader: M. Matsui).

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APPENDIX

Specimens of Tropidophorus grayi *from the Philippines examined for comparisons*

BMNH 1946.8.20.86–88 (syntypes of *Tropidophorus grayi* Günther 1861), BMNH 72.8.20.5–7; Luzon: MNHN 5443 (holotype of *Enoplosaurus insignis* Sauvage, 1870), MNHN 1900-350, BMNH 72.8.20.81, 95.11.7.12, SMF 15752–3, NSMT 03845; Cebu, SMF 15751; Unknown localities (probably Philippines): MNHN1997.3707–14, from a pet dealer.

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Red Variants of the Japanese Newt *Cynops pyrrhogaster* (Amphibia: Salamandridae): Review of Records and Captive Observations on the Heredity of Coloration

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Abstract: We surveyed information regarding the locality and detailed dorsal color pattern of red variants of the Japanese newt, *Cynops pyrrhogaster*. Results indicated that more individuals characterized by red dorsal ground color have been found in the western half of Japan and that such variants can be classified into three types. The heredity of coloration in one of these variant types, that having numerous small black spots on dorsum, was examined in a series of hybridization experiments in captivity. The results strongly suggested that coloration of this variant type is inherited and recessive.

Key words: Red variant; *Cynops pyrrhogaster*; Heredity; Japan; Recessive

INTRODUCTION

Cynops pyrrhogaster is an endemic Japanese newt distributed in most parts of Honshu, Shikoku, and Kyushu, including some adjacent islets (Nakamura and Uéno, 1963;

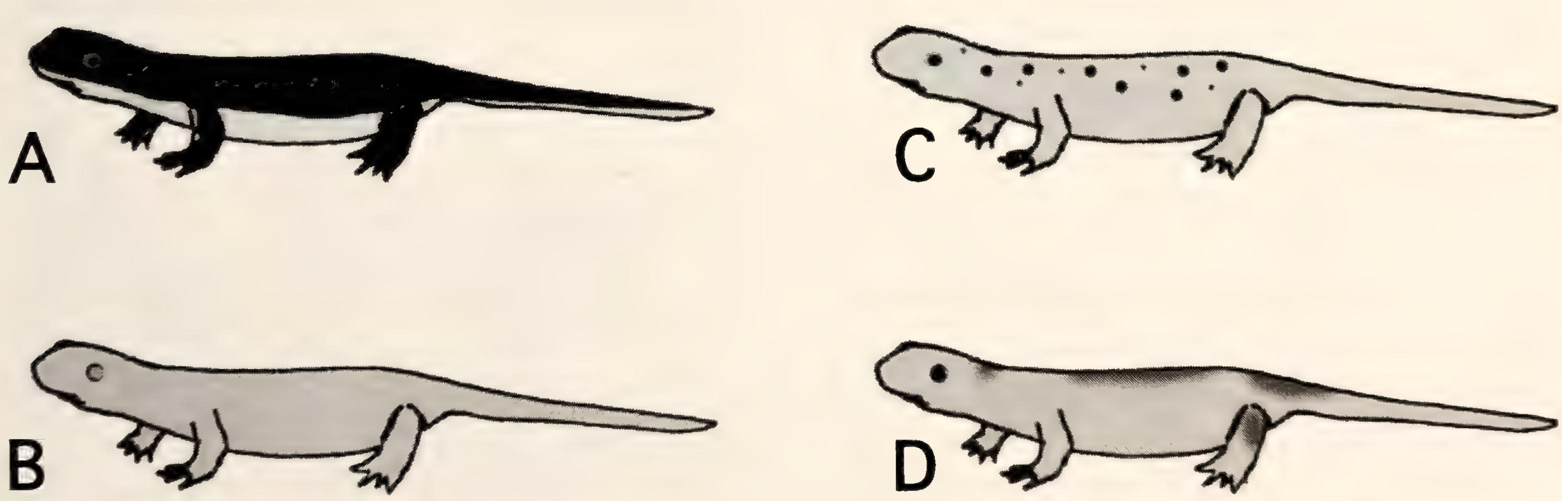

FIG. 1. Color variation in *Cynops pyrrhogaster*. A, normal type. B, type I. C, type II. D, type III. See text for further details on the latter three variant types.

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Uchiyama et al., 2002). Usually this newt has a uniformly black dorsum, which sharply contrasts with the brilliant red ground color of the venter (Fig. 1A). Besides the normal-colored individuals, red variants, characterized by reddish dorsal ground color, have occasionally been reported (e.g., Yamada and Kimura, 1985; Uchiyama et al., 2002). Nevertheless, very little work has been done on the variation in such variants or the mechanisms underlying their emergence.

In this study, we surveyed available information regarding occurrences of red variant *C. pyrrhogaster* in the past. We also examined the heredity of coloration characteristic of one of the variant color types by a series of captive hybridization. The results showed that the coloration of this variant type is inherited and recessive.

Materials and Methods

Localities and dorsal color patterns of red variant *C. pyrrhogaster* were surveyed on the basis of the literature, museum collections, and unpublished information provided by other colleagues through private communications. With respect to cases reported in newspapers, efforts were made to obtain pertinent information by locating corresponding specimens or by communicating with persons involved.

Animals used for the initial cross experiment included a male variant having a number of small dark spots against the red ground color on the dorsum (Fig. 2A). This individual was collected in 1993 in Kawauchi Town, Ehime Prefecture. Three females of normal coloration (Fig. 1A) collected from Shigenobu Town of the same prefecture in the same year were also used. Eggs from these females were artificially inseminated with dry sperm from the variant male in May 1994 and were allowed to develop and grow to sexual maturity in a terrarium (Matsui et al., 2002). Of a total of 14 F1 juveniles that metamorphosed, four (two males and two females) reached maturity by 2000, when they were put into two separate aquaria as male/female pairs in April. Three weeks later one of these F1 females laid eggs that had been naturally fertilized by sperm from her partner (see Matsui et al. [2002] for further details). Of the resultant F2 larvae, 11 successfully meta-

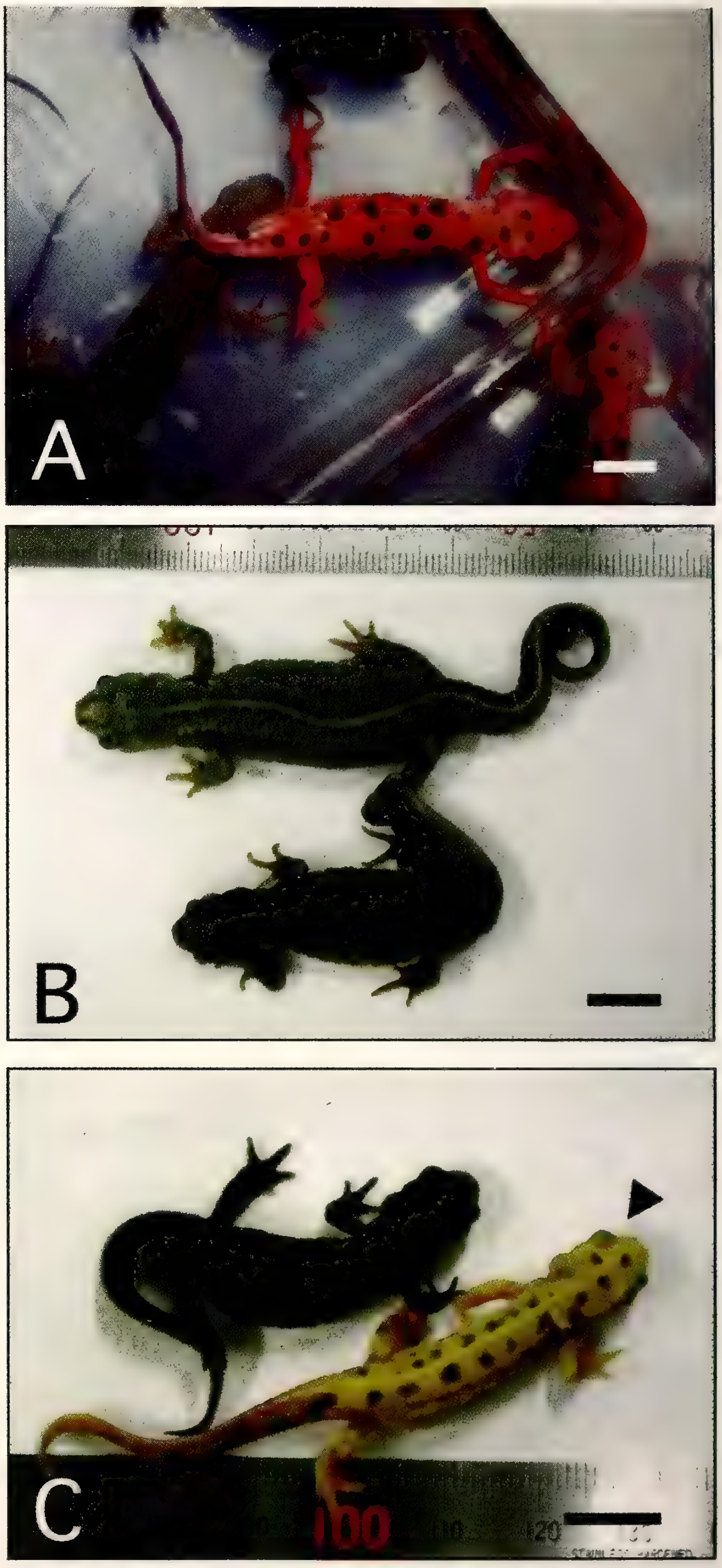

Fig. 2. Dorsal views of the *Cynops pyrrhogaster* examined in this study. A, male red variant used for the initial hybridization experiment. B, F1 individuals. C, F2 individuals. Arrow in "C" indicates an individual showing the red variant coloration. Scales equal 10 mm.

morphosed. The dorsal skin of each of these F2 juveniles was examined at metamorphosis under a stereoscopic microscope (Model SZX12, Olympus).

Results

Our survey detected a total of 19 red variants found in the past. Of the 18 of these variants for which locality data were available, 12, including six from Shikoku, five from western Honshu, and one from Kyushu, were from the western half of Japan. The remainder were from the central (n=2) or eastern part of Honshu (n=4) (Table 1).

On the basis of the dorsal color pattern, these red variants were classified into three distinct types—simple albino characterized by the absence of any dorsal marking (type I: see Fig. 1B), spotted variant characterized by numerous small black spots (type II: Fig. 1C), and cloudy variant characterized by a few large cloudy markings (type III: Fig. 1D). Numerically, type II was dominant (n=13 in Table 1), followed by type I (n=3), and type III (n=2). The red variant male used for the initial hybridization in the laboratory (Fig. 2A) belonged to type II.

All of the 14 F1 individuals had the normal uniformly black dorsum (Fig. 2B). However, the 11 F2 individuals included five with paler dorsal skin (Fig. 3A), and the remaining individuals had a normal dark dorsum (Fig. 3B), although none of these F2 individuals had

TABLE 1. List of red variants of the newt *Cynops pyrrhogaster* reported up to 2003. J, juvenile. M, male. F, female. See text and Fig. 1 for abbreviations of variant types.

	Variant type	Year	Locality	Sex/age	Source
1	Type I	1806	No data	No data	Kurimoto, 1811
2	Type II	1936	Kochi Town, Hiroshima Prefecture	F	Sato, 1943
3	No data	1937–1943	Shakuji River, Tokyo	No data	Kawakami, 1948
4	Type II	1962	Tottori City, Tottori Prefecture	M	*1
5	Type II	1976	Kayo Town, Okayama Prefecture	M	Matsumoto, 1979
6	Type III	1980	Tono City, Iwate Prefecture	Plur.	Yamada and Kimura, 1985
7	Type I	No data	Imajo Town, Fukui Prefecture	F	Sasaji, 1989
8	Type II	1993	Kawauchi Town, Ehime Prefecture	M	This study
9	Type II	1995	Yanadani Village, Ehime Prefecture	M	Omogo Mountain Museum, 1999
10	Type II	1995	Saihi Town, Nagasaki Prefecture	F	Matsuo and Matsumoto, 1995
11	Type II	1996	Omogo Village, Ehime Prefecture	M	Omogo Mountain Museum, 1997
12	Type III	1996	Fukushima Prefecture	M	Uchiyama et al., 2002
13	Type II	1997	Midori Town, Hiroshima Prefecture	F	*2
14	Type II	1997	Kainan City, Wakayama Prefecture	No data	Wakayama Nature Museum, 1997
15	Type II	1998	Asahi Town, Yamagata Prefecture	F	*3
16	Type II	1998	Uchiko Town, Ehime Prefecture	F	Omogo Mountain Museum, 1999
17	Type II	2001	Yaotsu Town, Gifu Prefecture	F	Yaotsu Town, 2001
18	Type I	2001	Tosa City, Kouchi Prefecture	J	*4
19	Type II	2002	Omogo Village, Ehime Prefecture	F	*5

*1 Deposited in the Tottori Prefectural Museum with catalogue no. 788-0101-01.
*2 I. Miura, personal communication; also see "http://labs.sci.hiroshima-u.ac.jp/homepage/amphibia/irogawari.html."
*3 Deposited in the Tochigi Prefectural Museum with catalogue no. TPM-Am2864.
*4 T. Fujitani, personal communication.
*5 K. Okayama, personal communication.

differed at all during the larval stage. Stereomicroscopic observations showed that the five light F2 variants had numerous minute melanophores on otherwise pink dorsal skin (Fig. 3C), whereas in the other six, melanophores were much more dense, forming large black spots all over the dorsal skin (Fig. 3D).

Two weeks after metamorphosis, these apparently variant and normal individuals took on colorations similar to those in the adult type II and normal individuals, respectively (Fig. 3E). The dorsal skin of an F2 variant at 32 months after metamorphosis is shown in Fig. 3F. At this stage, melanophores in the dorsal skin of the variant individual aggregated to form a smaller number of larger black spots. The

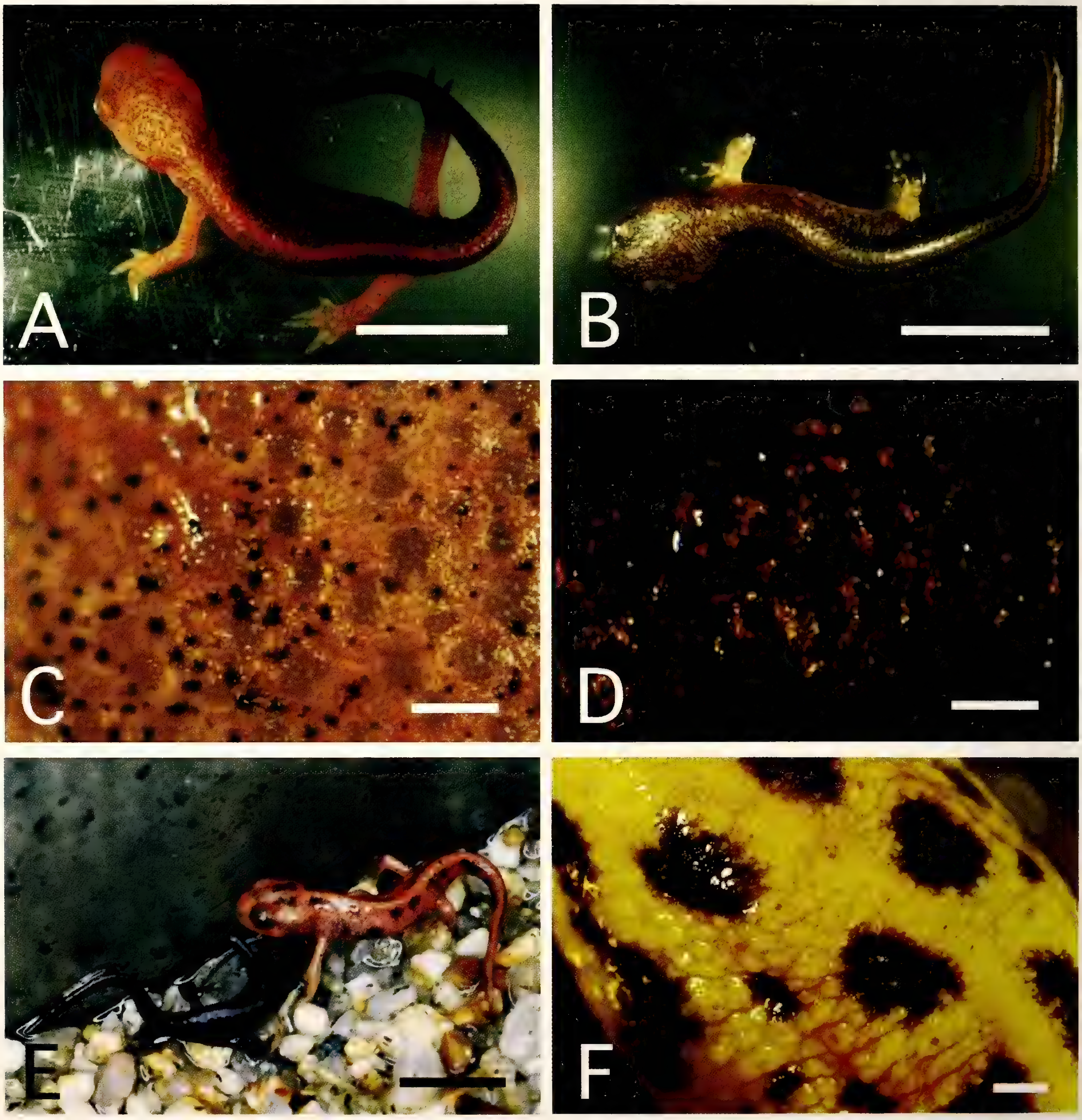

FIG. 3. F2 juveniles at and after metamorphosis. A, F2 individual with brownish coloration at metamorphosis. B, F2 individuals with normal coloration at metamorphosis. C, higher magnification of the dorsal skin of "A." D, higher magnification of the dorsal skin of "B." E, F2 juveniles at two weeks after metamorphosis. F, higher magnification of the dorsal skin of an F2 red variant at 32 months after metamorphosis. Scales equal 10 mm in "A," "B," "E" and "F," and 100 μm in "C" and "D."

dorsal ground color was much more yellowish than that of the same individual at metamorphosis (Fig. 3E) or of the original variant male (Fig. 2A).

Discussion

Sawada (1963), in a study of mating behavior in *C. pyrrhogaster*, tentatively defined six infraspecific races that might be reproductively isolated from each other through behavioral differentiation. On the other hand, Hayashi and Matsui (1988), based on allozyme analyses, provided a dendrogram depicting several genetically divergent groups of populations within this newt, but these genetic groups were not necessarily compatible with the races assumed by Sawada (1963). Currently available information on the red variants (Table 1) suggests that there is no strict correspondence between the emergence of these variants and the races recognized by Sawada (1963) or the genetic groups outlined by Hayashi and Matsui (1988). This suggests that mutations leading to the red variant coloration preceded the isolation or differentiation among the newt populations, although occurrences of independent mutations on the same gene or on different loci of multiple genes subsequent to such isolation or differentiation is also possible.

The results of our series of experimental hybridizations in the laboratory strongly suggest that in *C. pyrrhogaster* the dorsal skin coloration characteristic of the type II variant is inherited and recessive. The distinctly yellowish dorsal ground color exhibited by the captive raised variant (Fig. 3F) may be the consequence of carotinoid deficiency in the diet, as in the case of ventral coloration in this newt, which, although normally brilliant red, often turns to be more or less yellowish under captive condition (Matsui et al., 2002).

Further study is needed to clarify the genetic mechanisms responsible for the emergence of red variants (Fig. 1, Table 1). It would be of particular interest to examine whether the three types of variants reflect mutations at three different loci, as in the case of albinos in the tree frog *Hyla japonica* (see Nishioka and Ueda, 1977).

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Rainwater Drinking by Free-ranging Japanese Pitvipers, *Gloydius blomhoffii*

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Abstract: Three free-ranging gravid *Gloydius blomhoffii* were observed drinking drops of water from their skin or from leaves during a brief summer rain shower in Hokkaido, Japan. These behaviors may be common and important for gravid females, because they are sedentary and bask in areas with no nearby water otherwise.

Key words: Drinking rain drops; *Gloydius blomhoffii*; gravid females; free-ranging; pitviper

Although drinking of integumentally-captured water by captive snakes appears to be common (Louw, 1972; Robinson and Hughes, 1978; Miller, 1985; Somma, 1989; Andrade and Abe, 2000), rarely has this behavior been observed in free-ranging snakes (Robinson and Hughes, 1978; Miller, 1985; Greene, 1986; Ashton and Johnson, 1998). During the course of our field studies on *Gloydius blomhoffii* in Kaminokuni of the Hiyama region of Hokkaido, similar drinking behavior of gravid *G. blomhoffii* was observed in a small area, termed the basking spot, where gravid females aggregate. The snakes were captured, processed (measured, weighed, marked by nuchal painting and passive integrated transponder [PIT] tags, sexed by probing hemipenes, and assessed for female reproductive condition by ventral palpation and/or x-ray), and released at the exact point of capture within 1 h after capture.

Basking spot A, studied from 30 May to 27 October 2002, comprised an area of ca. 9 m^2 on a south-facing slope and received on 57 days of rain in a 151-day study period. The closest water source to basking spot A was ca. 30 m distant. At 1050 h on 20 October 2002, three gravid females were observed at basking spot A. The weather was sunny and air temperature was 24 C. Two snakes (557 mm in snout–vent length [SVL] and 264.2 g in body mass [BM], and 538 mm SVL and 235.0 g BM) were positioned in resting coils, basking in a sun/shade mosaic pattern created by vegetation. When it began to rain, both snakes uncoiled the anterior halves of their bodies and lowered their heads downhill from where they had been lying. The anterior portions of their bodies were ca. 35 degrees above the plane defined by the posterior portions of their bodies. Raindrops began to strike each snake's head at a rate of ca. 1 drop/sec and effectively flowed along a "downspout" formed by their now lower, anterior body portion. As drops collected on the anteriormost labial scales, the snakes drank them. Drinking bouts lasted ca. 190 seconds for each snake, after which time they raised their heads and recoiled into their original coiled posture. The third snake (600 mm SVL, 213.8 g BM) was observed drinking beads of water on a leaf. During the rain, she positioned her mouth so that she could drink the raindrops flowing down the leaf of a young *Actinidia arguta* growing along the ground. In all cases, drinking by the snakes ceased before the rain stopped.

The observed drinking behavior by *G. blomhoffii* may be common and important in the present population, especially among gravid females. First, daily movements of the gravid females were confined to an area of a few square meters within a basking spot (Sasaki, unpublished data). Thus, spatially close ponds and creeks may not be available for gravid females. Second, metabolic water obtained through feeding may not be available since feeding during pregnancy is rare (Sasaki, unpublished data). Considering the estimated low frequency of water intake by *Trimeresurus flavoviridis* of equivalent body size (ca. every 10th day: Nishimura and Kamura, 1992), drinking during rainy days in our study

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area may be sufficient for a snake's needs. Drinking of water from leaves was reported to be common among *G. blomhoffii* held in captivity (Central Research Laboratory, Yomeishu Seizo Co., Ltd., 1999).

Drinking of water from the skin has been suggested to be an adaptation for life in habitats with unpredictable and/or limited water availability (Louw, 1972; Somma, 1989; Greene, 1997; Andrade and Abe, 2000). A gravid *Bothrops asper*, for example, was observed drinking from her skin (Greene, 1986). For several weeks, this snake did not feed and her activities were confined to a few square meters. There was no standing water in her immediate environment. This natural history account is similar to ours. The full significance of drinking actions in these snakes, however, must await additional research.

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We thank Dr. Sen Takenaka for many helpful suggestions during this ongoing study. We also thank Aaron Place for useful comments on the manuscript and Dr. Tetsu Kawamata of the Kawamata Dog and Cat Clinic, Hakodate, Hokkaido, Japan, for his generous offer (free of charge) to take the x-rays of our snakes.

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of Ogasawara (Bonin) Islands, South Japan. Bull. Herpetol. Soc. Japan 2000: 4-7. (in Japanese with English abstract)
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Detailed information not essential to the text but important to subsequent evaluation (such as that regarding specimens examined) may be placed under the major heading, APPENDIX, and an appropriate sub-subheading. When institutional abbreviations are used in APPENDIX, and/or in the main text, it is recommended, as far as possible, to follow Leviton et al. (1985) or Leviton and Gibbs (1988) (see example of references above for detailed information on these publications) with an explicit statement in APPENDIX (or otherwise, in MATERIALS AND METHODS of the main text): e.g.,

APPENDIX

<u>Specimens examined</u>

Catalogue numbers of specimens deposited in the zoological collection of Kyoto University Museum are preceded by KUZ. The other acronyms are those suggested by Leviton et al. (1985).

<u>Geoemyda japonica</u>: Okinawajima, Okinawa Pref., Japan, KUZ R36720, NSMT H02083-02086; Kumejima, Okinawa Pref., Japan, KUZ R36721, OMNH-R3334. <u>G. spengleri</u>: Vietnam, NSMT H9999, ...

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$\bar{x}$ (mean), n (sample size), N (chromosome number, but see below for the use to refer to "north latitude"), no. (number), yr (year[s]), mo (month[s]), wk (week[s]), h (hour[s]), min (minute[s]), s (second[s]), P (probability), df (degrees of freedom), SD (standard deviation), SE (standard error), NS (not significant), l (liter), kg (kilogram), g (gram), m (meter), cm (centimeter), mm (millimeter), μm (micron), C (degrees Celsius, not °C), asl (above sea level; given as, e.g., 100 m asl), °, ', and " (degrees, minutes, and seconds in geography, respectively), N, S, E, and W (north and south latitudes, and east and west longitudes, respectively, but only when preceded by values with appropriate geographical units; e.g., 15°25'N, 121°43'E).

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FUTURE MEETING
Toho University (Narashino Campus), Funabashi, Chiba, Japan, 25–26 October 2003
(Sei-ichi Kohno, Chair)

Current Herpetology 22(2): 51–60, December 2003

Two New Species of the Genus *Philautus* (Anura: Rhacophoridae) from the Western Ghats, Southwestern India

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Abstract: Two new species of the bushfrog genus *Philautus* are described from the Western Ghats, southwestern India. *Philautus luteolus* is a medium-sized (ca. 27 mm SVL in males) yellowish frog with a few indistinct markings. From the most similar species *P. travancoricus*, *P. luteolus* can be distinguished by the lack of conspicuous black markings and by the rather pointed longer snout. Advertisement calls are long trills consisting of a short introductory phase with fast-repeated pulses and a long main phase with slowly repeated pulses. *Philautus tuberohumerus* is a tiny (ca. 18 mm SVL in males) dark brown frog and its humerus bone has a unique conspicuous tubercle projecting antero-ventrally. From the similar species *P. bombayensis*, it is easily distinguishable by its distinctly small size and absence of a papilla on the tongue. Advertisement calls are sharp, metallic sounds composed of short pulses which are repeated very slowly.

Key words: New species; *Philautus luteolus*; *Philautus tuberohumerus*; Rhacophoridae; Western Ghats, India

INTRODUCTION

Recently, a remarkable amphibian diversity has been discovered in Sri Lanka (Dutta and Manamendra-Arachchi, 1996; Pethiyagoda and Manamendra-Arachchi, 1998; Meegaskumbura et al., 2002). Especially abundant are rhacophorid treefrogs, and Pethiyagoda and Manamendra-Arachchi (1998) estimated that about 200 species remain to be described as new. This is largely due to the fact that little exploration has been done in the forest layers.

We think that the same is true for the anuran fauna of the Western Ghats, southwestern India. As Inger and Dutta (1986) stressed, Indian amphibian species and genera are most adundant in the Western Ghats of the West Peninsular region (Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala). In this region, a total of 17 genera and 99 species of anurans and four genera and 13 species of gymnophionans were listed by Inger and Dutta (1986), and Daniels (1992) listed 103 anuran and 14 gymnophionan species, most of them endemic to the Western Ghats. In the Western Ghats, new species have been described year after year (e.g. Das and Ravichandran, 1998; Vasudevan and Dutta, 2000; Bossuyt, 2002), suggesting that systematic and comprehensive herpetological surveys are still needed.

The majority of rhacophorid treefrogs in the

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Western Ghats are members of the bushfrog genus *Philautus*. Inger and Dutta (1986) listed 22 *Philautus* species in this region. Of these, Bossuyt and Dubois (2001) transferred four species to the genus *Micrixalus* and one to the genus *Rhacophorus*, put one species in the synonymy of an *Indirana* species and four species in the synonymy of other *Philautus* species, removed *P. leucorhinus* by restricting its range to Sri Lanka, and revived two names (*Philautus tinniens* and *Philautus wynaadensis*) created by Jerdon (1853). Finally, Bossuyt (2002) described *P. griet* from Mannar, Kerala. Thus, a total of 14 *Philautus* species are currently recognized as occurring in the Western Ghats.

We collected two *Philautus* species, all males, from several localities in the Western Ghats which did not fit any previous descriptions. These are named here as new species.

Materials and Methods

Specimens were collected from Malleshwaram in Kudremukh, Chikmagalur district (13°12'N, 75°16'E), Madenadu near Medikeri, Kodagu district (12°27'N, 75°38'E), and Kirundadu near Madikeri (12°29'N, 75°47'E), all in the southern part of Karnataka state, during the breeding seasons from 1999 to 2003. Measurements were made on preserved specimens (type series and several specimens deposited in Rondano Bio-diversity Research Laboratory (RBRL), St. Aloysius College) using digital calipers under a head magnifying glass.

Abbreviations of the measured parts are: SVL (snout–vent length), HL (head length, from tip of snout to angle of jaws), HW (head width, at angle of jaws), SN (distance from tip of snout to nostril), IN (internarial distance), NE (distance from nostril to anterior corner of eye), ED (horizontal eye diameter), IO (interorbital distance, narrowest distance between upper eyelids), UL (width of upper eyelid), TYD (horizontal tympanum diameter), FHL (forearm and hand length, from elbow to tip of longest finger), HAL (hand length, from base of outer palmar tubercle to tip of longest finger), FIL1–FIL4 (length of no. 1–4 finger, from junction of no.n and no.(n–1) finger to tip of no. n finger, FIL1 being measured from junction of no. 1 and no. 2 fingers), FD1-FD4 (disk width of no. 1–4 finger), HLL (hind limb length, from vent to tip of longest toe), FL (femur length, from vent to knee), TL (tibia length, from knee to ankle), TFL (tarsus and foot length, from ankle to tip of longest toe), FOL (foot length, from base of inner metatarsal tubercle to tip of longest toe), TOL1–TOL5 (length of no. 1–5 toe, measured similarly as in finger length), TD1–TD5 (disk width of no. 1–5 toe), IMT (length of inner metatarsal tubercle).

Because we found that one of the two new species had unique humerus bones, we made glycerin-cleared skeleton preparations from one specimen using the method of Klymkowsky and Hanken (1991). In this method bone is stained red by Alizarin Red S and cartilage is stained blue by Alcian Blue 8GX.

Philautus luteolus sp. nov.

Figs. 1 and 2., Table 1

Philautus species D: Kuramoto and Joshy, 2000.

Philautus cf. *travancoricus*: Kuramoto and Joshy, 2001.

Holotype

BNHS 4191 (Bombay Natural History Society), adult male collected by M. Kuramoto from Kirundadu, Kodagu, Karnataka, ca. 920 m in altitude on 16 August 2001.

Paratypes

BNHS 4192, adult male collected by S. H. Joshy from Kudremukh, Chikmagalur, Karnataka, ca. 940 m in altitude on 10 June 2003. OMNH Am 11412 (Osaka Museum of Natural History), adult male collected by M. Kuramoto from Kudremukh on 5 July 1999.

Diagnosis

Medium-sized *Philautus* (SVL ca. 27 mm in

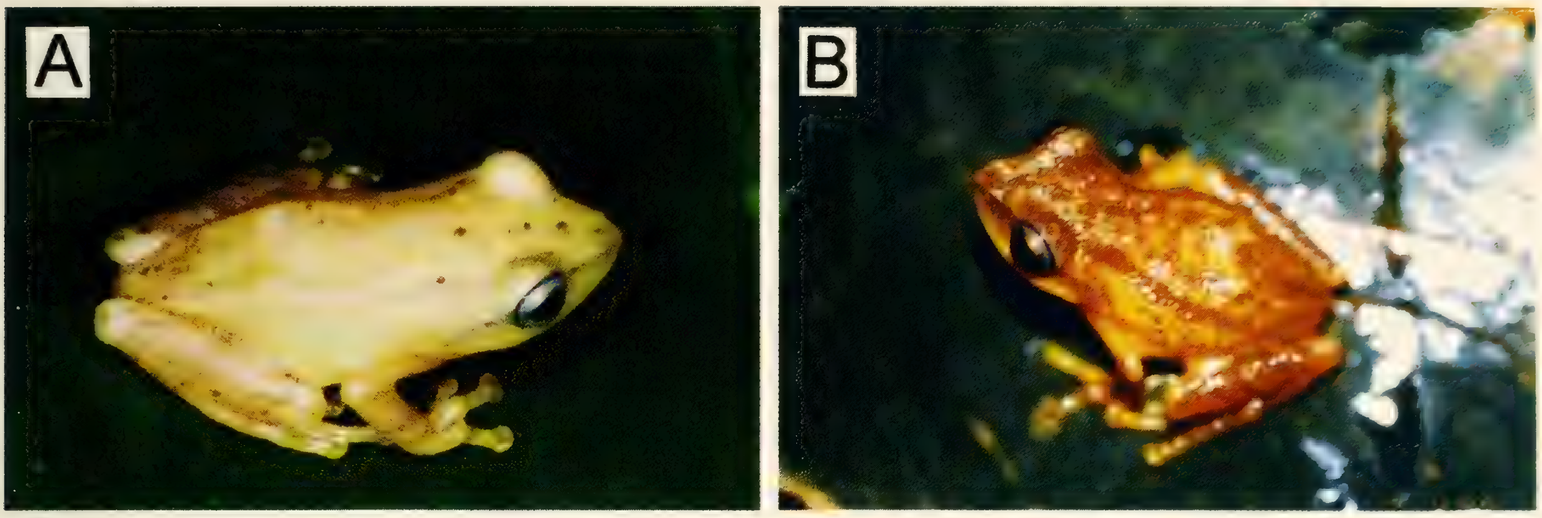

FIG. 1. *Philautus luteolus*. A: Holotype BNHS 4191 from Kirundadu. B: Darker color phase observed in a specimen from Kudremukh during the daytime (specimen number not specified).

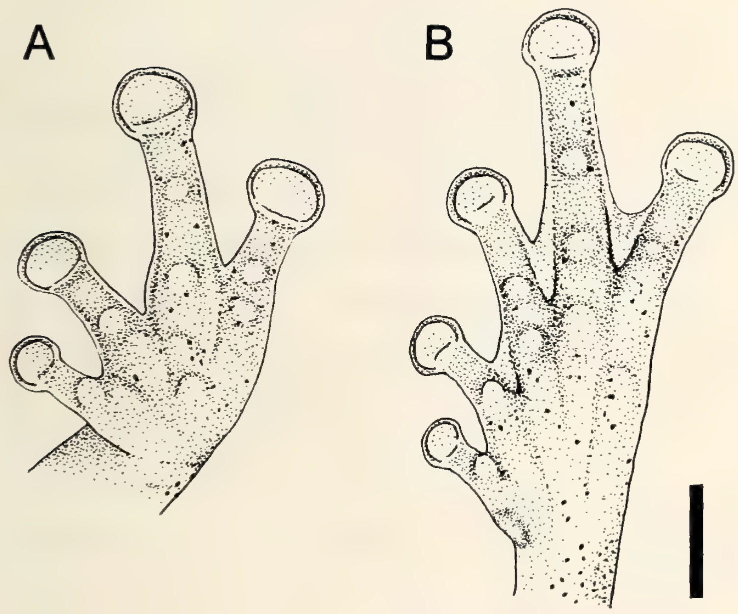

FIG. 2. Ventral view of left hand (A) and left foot (B) of *P. luteolus*. Scale 2 mm.

males) with yellow to yellowish brown dorsal coloration lacking distinct markings. This coloration readily distinguishes this species from other *Philautus* species in the Western Ghats. *Philautus luteolus* lacks black streaks on both sides of anterior dorsum or a few large black spots on dorsum and tibia which are present in the most similar species, *P. travancoricus*. The snout of *P. travancoricus* is round and its length is equal to eye diameter, whereas the snout of *P. luteolus* is rather pointed and longer than eye diameter. Advertisement calls are long trills with an initial short phase consisting of fast-repeated pulses followed by a long main phase consisting of slowly repeated pulses.

Description of the type series (three males)

Body moderately elongated. Head wider than long. Canthus rostralis distinct. Loreal region slightly concave. Snout slightly pointed, projecting beyond mouth. Nostril nearer to tip of snout than to eye. Eye diameter subequal to interorbital distance, the latter much larger than upper eyelid width. Tympanum small and indistinct. Supratympanic fold distinct. No papilla on tongue.

Hand longer than forearm. Finger length 3>4≥2>1 (means of the type series in mm: FIL1=2.21, FIL2=3.37, FIL3=4.84, FIL4=3.43; Table 1, Fig. 2). Finger disc large with distinct circummarginal groove (means of the type series in mm FD1=1.00, FD2=1.28, FD3=1.59, FD4=1.50). No web between fingers. Tibia subequal to femur. Toe length 4>3>5>2>1 (means of the type series in mm: TOL1=2.01, TOL2=3.22, TOL3=4.23, TOL4=6.70, TOL5=4.16). Toe disc with circummarginal groove, slightly smaller than finger disc (means of the type series in mm: TD1=0.93, TD2=0.98, TD3=1.15, TD4=1.36, TD5=1.36). Toes 1/3 webbed (Fig. 2). Inner metatarsal tubercle small and indistinct. No tarsal fold. Heels touch or scarcely overlap when legs folded at right angle to the body, and reaches eye when stretched forward along body axis.

Dorsal surface and upper surface of limbs finely granulated without distinct ridges. Venter finely granulated. An external subgular vocal

TABLE 1. Measurements of male specimens of *Philautus. luteolus* (in mm).

	Holotype BNHS 4191	Paratype BNHS 4192	Paratype OMNH Am 1141	Type series plus two RBRL specimens (n=5) x̄±SD (Min–Max)
SVL	27.8	26.7	24.7	26.54±1.52 (24.7–28.1)
HL	7.7	7.5	7.5	7.48±0.20 (7.1–7.7)
HW	10.2	9.9	9.4	9.69±0.36 (9.3–10.1)
SN	2.0	2.1	1.9	1.91±0.15 (1.8–2.1)
IN	2.8	2.7	2.4	2.59±0.21 (2.3–2.8)
NE	3.7	2.4	2.4	2.84±0.57 (2.4–3.7)
ED	3.8	3.2	3.4	3.50±0.23 (3.2–3.8)
IO	3.4	2.8	3.3	3.35±0.40 (2.8–3.9)
UL	2.5	2.6	2.4	2.32±0.25 (2.0–2.6)
TYD	1.4	0.8	1.2	1.19±0.26 (0.8–1.4)
FHL	13.6	13.8	12.2	12.98±0.81 (12.1–13.8)
HAL	7.5	7.1	7.2	7.21±0.17 (7.1–7.5)
HLL	37.5	37.6	35.3	36.89±1.84 (34.8–39.3)
FL	13.2	13.0	12.6	12.66±0.79 (11.3–13.2)
TL	13.5	12.8	12.4	12.69±0.66 (11.7–13.5)
TFL	17.7	17.7	16.5	17.13±0.95 (15.8–18.0)
FOL	10.6	10.5	9.8	9.96±0.53 (9.3–10.6)
IMT	0.9	0.8	1.2	0.94±0.17 (0.8–1.2)

sac. No bony process on humerus bone.

Coloration

In life (Fig. 1), upper surface uniformly pale yellow or brownish yellow with a few small brown spots mostly along lines from snout to vent and from eye to vent. Leg with faint cross bars or a few brown spots. Iris pale gold encircled with black. Venter and vocal sac uniformly light yellow. Usually, color is pale yellow at night and becomes brownish in the daytime.

In preservative, dorsum and upper surface of limbs pale yellow to yellowish brown with slightly dark, irregular, longitudinal series of small indistinct patches. Indistint dark bars on limbs. Indistinct dark line from tip of snout, through nostril and eye, to the base of arm. Lower surface whitish without any markings.

Advertisement calls

The advertisement calls (Kuramoto and Joshy, 2001) are long trills (often exceeding 1 min) with an initial short phase composed of fast-repeated pulses and a long main phase of slowly repeated pulses (3–4 pulses per sec). The dominant frequency is about 2.7 kHz. The males call from branches or leaves in secondary forest and bush by the roadside at night. Calling sites are usually more than 1.5 m above ground, and we could not find any water bodies around the collecting sites.

Etymology

From Latin *luteolus* meaning yellowish, indicating the body color of this frog.

Comments

This species fits the original description of *Philautus travancoricus* by Boulenger (1891; as *Ixalus travancoricus*) except that our spec-

imens lacked "larger black dots scattered on the back and on the tibia", "a blackish streak on each side of the anterior half of the back", and "a narrow band of pigment along the upper surface of the femur". He also described the skin of *P. travancoricus* as "smooth", which does not apply to the present species. Dorsal color was described as "cream", apparently based on the preserved type specimen (a female from Bodanaikanur (=Bodinaikenur), Tamil Nadu; SVL 31 mm); color in life is unclear, but it may be yellowish. Except for this uncertain example, no other *Philautus* species in the Western Ghats has yellow or yellowish dorsal coloration.

The snout of *P. travancoricus* was reported to be round and its length equal to eye diameter. However, the snout of *P. luteolus* is rather pointed and much longer than eye diameter (Table 1). *Philautus luteolus* is smaller than *P. travancoricus* (SVL ca. 27 mm vs. 31 mm), but this difference may be attributable to sexual difference because *P. travancoricus* was described on the basis of a single female specimen.

Inger et al. (1984) noted a conspicuous color change in *P. femoralis* from Ponmudi, Kerala; it changes color from green to yellow. However, the dorsal color was reported to be invariably green when collected (undoubtedly at night). In *P. luteolus*, the dorsal color is yellowish day and night, and the yellowish color changes only slightly.

Philautus luteolus occurs in Kudremukh, Manenadu (ca. 1120 m in altitude) and Kirundadu but does not occur in coastal lowland around Mangalore (12°52'N, 74°51'E). We have not found any females.

Philautus tuberohumerus sp. nov.
Figs. 3 and 4, Table 2

Philautus species C; Kuramoto and Joshy, 2000.

Philautus cf. *bombayensis*: Kuramoto and Joshy, 2001.

Holotype

BNHS 4193, adult male collected by S. H. Joshy from Kudremukh, Chikumagalur, Karnataka, ca. 940 m in altitude on 15 June 2000.

Paratypes

BNHS 4194, adult male collected by M. Kuramoto from Kudremukh on 15 June 2000. OMNH Am 11413, adult male collected by M. Kuramoto from Kudremukh on 15 June 2000. OMNH Am 11414 (cleared skeleton), adult male collected by S. H. Joshy from Kudremukh on 15 June 2000.

Diagnosis

A small frog (SVL ca. 18 mm in males) with brown to dark brown dorsal color and conspicuous yellow spots on the anterior surface of the thigh. A bony projection on the underside of the humerus bone, which is unique to this species. From the most similar species *P. bombayensis* it is clearly distinguishable by its distinctly small size and absence of a papilla on the tongue. Advertisement calls are composed of a series of slowly repeated sharp pulses.

Description of the type series (three males)

Body rather robust. Head broader than long. Snout slightly pointed. Canthus rostralis rounded. Loreal region slightly concave or flat. Nostril nearer to tip of snout than to eye, and protruding beyond the line between tip of snout and anterior corner of eye when viewed from above. Interorbital distance much wider than upper eyelid. Tympanum small and indistinct, less than half eye diameter. Supratympanic fold distinct. No papilla on the tongue.

Hand longer than forearm. Small knob-like bony projection on the antero-ventral side of the humerus bone, as indicated in a cleared skeletal preparation (OMNH Am 11414: Fig. 3D). This projection is not obvious externally, but readily recognizable by stroking the lower side of the upper arm with thin forceps or a needle. Finger length $3>2\geq4>1$(means of the type series in mm: FIL1=2.03, FIL2=2.34, FIL3=3.36, FIL4=2.10). Fingers with a

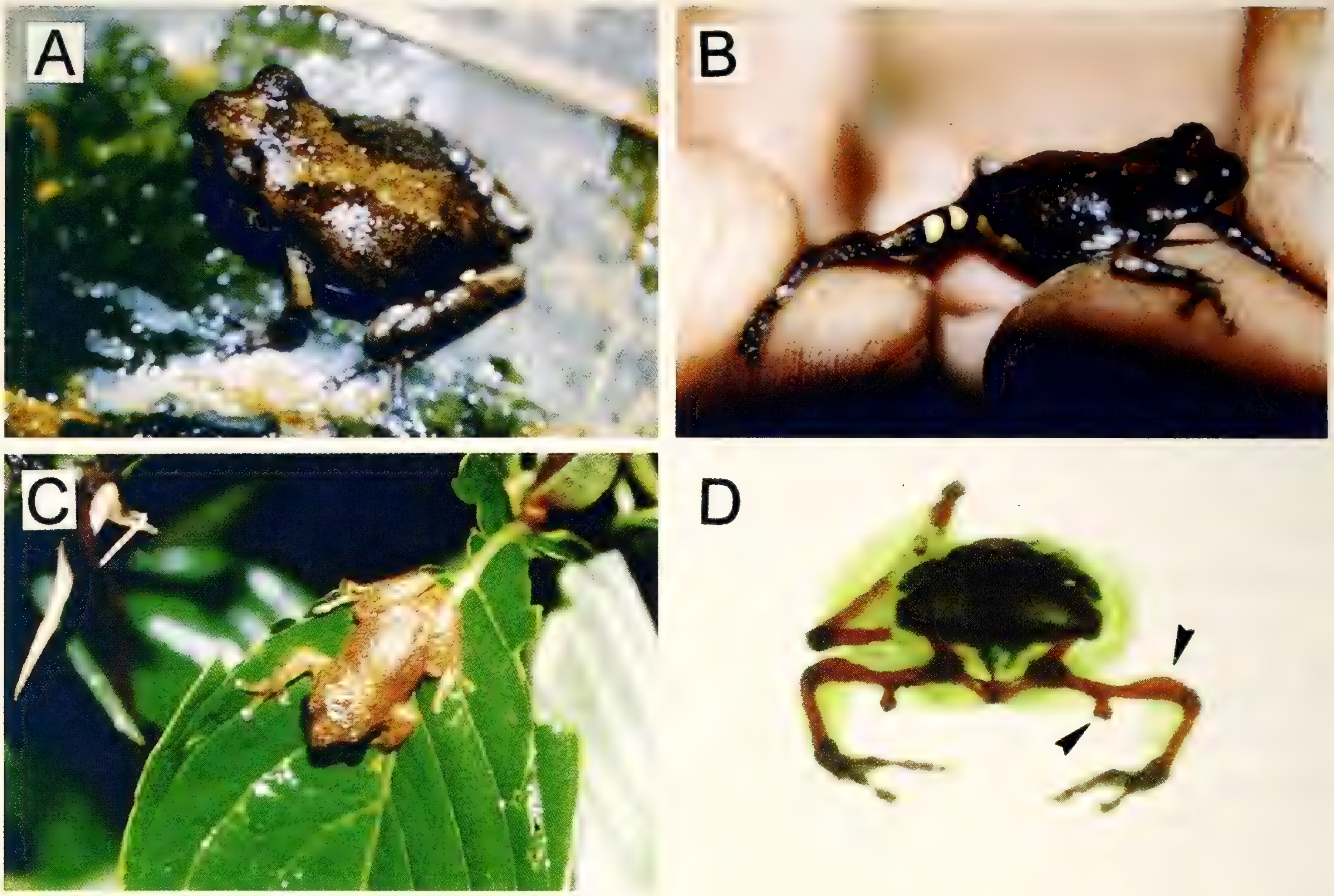

FIG. 3. *Philautus tuberohumerus*. A: Color phase during daytime. B: Showing yellow markings on thigh and groin. C: Color phase at night (A–C: Specimen numbers not specified). D: Anterior view of stained skeleton (OMNH Am 11414). Note the bony projection on the antero-ventral side of the humerus and the thick ends of the humerus (arrows).

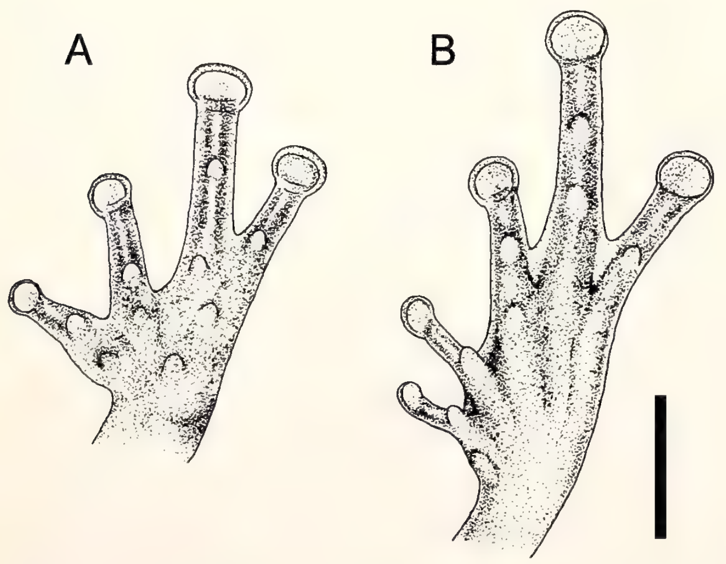

FIG. 4. Ventral view of left hand (A) and left foot (B) of *P. tuberohumerus*. Scale 2 mm.

large disc which has a distinct circummarginal groove (means of the type series in mm: FD1=0.51, FD2=0.66, FD3=0.96, FD4=0.90). No web between fingers. Subarticular and palmar tubercles well developed (Fig. 4). Femur slightly longer than tibia. Toe length 4>3≥5>2>1 (means of the type series in mm: TOL1=1.44, TOL2=2.07, TOL3=2.92, TOL4=4.10, TOL5=2.89). Toe discs large, with a circummarginal groove (means of the type series in mm: TD1=0.54, TD2=0.66, TD3=0.76, TD4=0.88, TD5=0.74). Rudimentary web between toes (Fig. 4). Inner metatarsal tubercle moderate. No outer metatarsal tubercle. No tarsal fold. Heels overlap slightly when the legs are folded at right angles to the body, and reach the posterior corner of the eye when stretched forward along the body axis.

Dorsal surface rather coarsely granulated. A pair of wide ridges from the posterior corner of the eye, obliquely running toward the body axis, and extending parallel to the level of the arm base. Lower surface finely granulated. An

TABLE 2. Measurements of male specimens of *Philautus tuberohumerus* (in mm).

	Holotype BNHS 4193	Paratype BNHS 4194	Paratype OMNH Am 11413	Type series plus five RBRL specimens (n=8) x̄±SD (Min–Max)
SVL	18.2	17.4	17.9	17.84±0.56(17.3–19.0)
HL	4.5	5.5	5.2	4.85±0.36 (4.4–5.5)
HW	6.6	6.7	6.9	6.33±0.44 (5.5–6.9)
SN	1.1	1.2	1.6	1.24±0.15 (1.1–1.5)
IN	1.8	1.9	2.3	1.70±0.33 (1.4–2.3)
NE	1.4	1.0	1.6	1.48±0.23 (1.0–1.7)
ED	2.3	2.3	2.3	2.31±0.14 (2.1–2.3)
IO	2.4	2.4	2.6	2.46±0.24 (2.0–2.7)
UL	1.7	1.7	1.6	1.73±0.13 (1.5–1.9)
TYD	1.0	0.8	1.0	0.86±0.11 (0.7–1.0)
FHL	8.5	8.0	9.3	8.36±0.61 (7.5–9.3)
HAL	4.5	5.3	5.0	4.91±0.36 (4.5–5.6)
HLL	24.0	22.5	25.1	23.69±1.31 (21.7–25.2)
FL	9.3	8.2	9.0	8.44±0.64 (7.7–9.3)
TL	9.0	8.0	8.9	8.21±0.64 (7.2–9.0)
TFL	11.6	10.6	12.3	11.20±0.61 (10.6–12.3)
FOL	7.1	6.7	7.5	6.94±0.38 (6.3–7.5)
IMT	0.8	1.0	1.0	0.88±0.15 (0.6–1.0)

external subgular vocal sac.

Coloration

In life (Fig. 3), brown to dark brown, sometimes with pale or dark hourglass pattern or hourglass-like stripe from eyes to vent. Dark bar between eyes often present. Large yellow or reddish yellow markings near groin (Fig. 3B), which fade to white in preserved specimens.

In preservative, upper surface dark brown, leg with a few darker bands. Throat mottled with dark brown. Venter with numerous, large or small, whitish markings on dark brown. Undersurface of thigh and posterior surface of arm with small whitish spots. Large whitish markings on the posterior part of flank and anterior side of femur.

Color changes to some extent; during the daytime dorsal color becomes darker and dorsal markings such as the hourglass pattern and interorbital stripe tend to become more conspicuous than at night (Fig. 3A–C).

Advertisement calls

The advertisement calls (Kuramoto and Joshy, 2001) are a series of slowly repeated (1–2 sec interval) short pulses with a dominant frequency of about 4 kHz. Males call from among lower vegetation, often less than 50 cm above ground. Around the collecting sites, we could not find water bodies suitable for the life of aquatic larvae, suggesting direct development of this species as observed in *P. variabilis* (Patil and Kanamadi, 1997).

Etymology

Latin meaning "knobbed humerus", the unique characteristic of this frog.

Comments

This species fits the original description of

P. bombayensis from Castle Rock, Karnataka (Annandale, 1919; as *Ixalus bombayensis*) fairly well. However, *P. bombayensis* was reported to be 30 mm in SVL and to have a papilla on its tongue (Annandale, 1919; Ahl, 1931). In the present species the male frogs are much smaller than the value given for *P. bombayensis* (including males) and the tongue lacks a papilla.

The very sharp and loud calls of this species resemble the calls of *P. variabilis* described by Daniels (1998). He expressed the call of *P. variabilis* as a loud metallic "tink; tink-tink". Although this description of the call agrees with the call of the present species, the smaller body size and rudimentary webbing clearly discriminate this species from *P. variabilis*. Recent acoustic analyses (Kanamadi et al., 2001) revealed that the advertisement calls of *P. variabilis* begin with a few single note calls followed by multiple (2–19) note calls. Such a remarkable change in call types was not observed in *P. tuberohumerus*. *Philautus variabilis* from Ponmudi, Kerala is much larger than *P. tuberohumerus*, SVL being reported as 27 and 28.5 mm in males (Inger et al., 1984).

The peculiar projection on the upper arm is bony as revealed by skeletal preparations (Fig. 3D). Both ends of the humerus are very thick. We noticed that the arms were folded under the chest when the frog was anesthetized, so tightly folded that it was hard to extend them. Whether these characters are male-specific or not is unknown because no female specimens were obtained. Among the many specimens of *P. bombayensis* deposited in BNHS, there are small ones which resemble *P. tuberohumerus* in size. However, these do not have a bony knob on the underside of the humerus so far as determined by stroking. We could not examine whether these were males or juveniles. Günther (1858) examined osteological characters (apparently based on skeletal preparations) in the original description of *P. variabilis* (as *Ixalus variabilis* from Sri Lanka), but did not mention any peculiar bony process on the humerus, a conspicuous character that could not be overlooked.

We observed this species in Kudremukh and Kirundadu, but did not find it in coastal lowland around Mangalore. We have not found any females.

Discussion

Frost (2002) listed a total of 85 species in the genus *Philautus*. From geographic distribution, these can be divided into three groups. The first group consists of 31 species in the Philippines, Borneo, Java, Sumatra, and Malaya; the second consists of 26 species in the mountainous regions of Vietnam, Thailand, Myanmar, China, northeast India, Bhutan, and Nepal; and the third consists of 28 species in south India and Sri Lanka. Apparently these three groups have long been isolated from each other, and it is probable that many species have diverged within each region. In India, northeastern and southern distribution ranges are widely separated. Although there should be a phylogenetic relationship between the two Indian groups, many species of the Western Ghats may have diverged within this mountain range.

Phylogenetic relationships between species of the genus *Philautus* are poorly known. Bossuyt and Dubois (2001) placed two Bornean species in the subgenus *Gorhixalus* and seven Sri Lankan species in the subgenus *Kirtixalus*, thus implying close species relationships within the subgenera, but the relationships among the remaining species (subgenus *Philautus*) are obscure. Some authors (e. g. Emerson et al., 2000; Marmayou et al., 2000) used a few *Philautus* species from southeast Asia to construct a phylogenetic tree by DNA sequencing, but their results provided no information about species relationships within the genus *Philautus* as a whole. Meegaskumbura et al. (2002) constructed a tree from mitochondrial DNA sequencing data, in which three Indian, three southeast Asian, and many (mostly undescribed) Sri Lankan *Philautus* species were involved. From this tree, it became apparent that southeast Asian species (from

Borneo and Java) constitute a separate cluster from that of Sri Lankan and Indian species. Of the three Indian species (from the Western Ghats), *P. charius* and *P. signatus* form a cluster of their own, whereas *P. wynaadensis* form another cluster together with Sri Lankan species. More extensive comparisons are needed to elucidate species relationships in the genus *Philautus*.

Species identification and discrimination are difficult in the genus *Philautus* (Inger and Stuebing, 1997, p. 163). Many morphological features are common to many species and morphometric features are rather trivial for these small frogs. Inger et al. (1984) ascribed the discrepancy between their materials and the original descriptions to geographic variation. In fact, variations seem extensive regarding size, ratio of body parts, coloration, and so on. Several species of south India were originally described on the basis of frogs obtained from Sri Lanka (e. g., *P. femoralis*, *P. temporalis,* and *P. variabilis*), but no detailed comparisons between Indian and Sri Lankan populations have been made. Under these circumstances, it is necessary to accumulate precise data especially on live specimens such as coloration and acoustic features.

Many frog species in the Western Ghats show considerable color changes as exemplified by the work of Inger et al. (1984). We observed that *P. wynaadensis* changes dorsal color from reddish brown (at night) to pale gray or brownish gray (in the daytime). Although color changes are very common in many rhacophorid species, we noticed some remarkable color changes in other frogs. For example, a characteristic symmetric dark pattern on the back of *Microhyla ornata* from the Western Ghats disappears at night; instead, it becomes uniform dark red without showing any traces of symmetric pattern. For identification in the field, it is necessary to record the range of individual color variation.

Also important is to characterize a species by its acoustic features. To date, call structures of only four *Philautus* species are available in the Western Ghats (Patil et al., 1996; Kanamadi et al., 2001; Kuramoto and Joshy, 2001). Detailed examination of the call repertoires, advertisement, territorial, and warning calls, are needed. We expect that the combined set of these morphological and acoustic data from live specimens, together with morphometric data from preserved specimens and DNA sequencing, will aid in clarifying the rather confusing taxonomic status of the genus *Philautus*.

Krishnamurthy and Hussain (2000) listed four *Philautus* species (*P. charius*, *P. femoralis*, *P. glandulosus*, and *P. leucorhinus*) in Kudremukh, of which we found only *P. leucorhinus* (the populations should now be referred to as *P. wynaadensis*). We could not record calls of *P. femoralis* and *P. glandulosus* in Kudremukh. It is probable that some species confined themselves to particular kinds of habitat, thus segregate themselves from each other in microhabitat, as in Bornean species (Inger and Stuebing, 1997, p. 163).

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A New Subspecies of the Agamid Lizard, *Japalura polygonata* (Hallowell, 1861) (Reptilia: Squamata), from Yonagunijima Island of the Yaeyama Group, Ryukyu Archipelago

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Abstract: *Japalura polygonata*, occurring in the East Asian islands, is currently divided into three subspecies—*J. p. polygonata* from the Amami and Okinawa Groups of the central Ryukyus, *J. p. ishigakiensis* from the Miyako and Yaeyama Groups of the southern Ryukyus, and *J. p. xanthostoma* from northern Taiwan. A new subspecies is described for this species from Yonagunijima Island of the Yaeyama Group. This subspecies differs from other conspecific subspecies in having distinctly enlarged and irregularly arranged scales on the dorsolateral surface of the body. In other subspecies, the degree of enlargement of such scales is smaller, and they usually form somewhat regular rows in a transverse direction on the flanks, and in a longitudinal direction in the paravertebral region. Males of the present subspecies differ from those of other subspecies in having a series of large white spots against a dark grayish tan on the dorsolateral surface of the body, whereas the females are characterized by brilliant green dorsal coloration.

Key words: *Japalura polygonata*; New subspecies; Reptilia; Geographic variation; Yonagunijima Island; Ryukyu Archipelago

INTRODUCTION

The agamid genus *Japalura* consists of 24 species and two subspecies distributed from northern India and Nepal to the East Asian islands (Ota, 2000a,b; Gau and Hou, 2002: but see Marcey et al., [2000] for the possible non-monophyly of the western and eastern components, and Schleich and Kästle [2002] for a different generic arrangement). Of the species of *Japalura*, *J. polygonata* occupies the northeasternmost extremity of the geographic range of the genus (and actually of the family Agamidae as well), being distributed in northern Taiwan and most islands of the Ryukyus south of the Tokara Group. Three subspecies are currently recognized for the species—*J. p. polygonata* from the Amami and Okinawa Groups of the central Ryukyus, *J. p. ishigakiensis* from the Miyako and Yaeyama Groups of the southern Ryukyus, and *J. p. xanthostoma* from northern Taiwan (Ota, 1991). In the process of their infraspecific classification, however, very few island samples have been examined. Hallowell (1861), for example, described the species (as *Diploderma polygonatum*) only on the basis

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of a single specimen from "Amakarima Island" (subsequently restricted to the Kerama Islands of the Okinawa Group by Stejneger [1907]). Van Denburgh (1912), in describing two subspecies, *J. p. ishigakiensis* and *J. p. miyakensis*, examined specimens only from Ishigakijima and Iriomotejima Islands for *ishigakiensis*, Miyakojima Island for *miyakensis,* and Okinawajima and Amamioshima Islands for the nominotypical subspecies. Although several subsequent authors referred to populations of *J. polygonata* on some other islands (e.g., Okada, 1937; Nakamura and Uéno, 1963; Ikehara et al, 1984; Toyama, 1985), or proposed infraspecific classification different from that proposed by Van Denburgh (1912) (Nakamura and Uéno, 1963; Matsumoto, 1979; Ota, 1991), none of them investigated between-island variation in *Japalura polygonata* in detail.

Yonagunijima Island (Fig. 1), a component of the Yaeyama Group, is the westernmost island of the Ryukyus. Its herpetofauna, while sharing most species and subspecies exclusively with other southern Ryukyu islands (Yasukawa et al., 1996; Toda et al., 1997; Ota, 1998, 2000c; Chen et al., 2001), includes two endemic snakes (*Calamaria pavimentata miyarai* and *Elaphe carinata yonaguniensis*), whose closest relatives occur only in Taiwan and the southern part of the continent (Takara, 1962; Ota, 1998, 2000c). The occurrence of *Japalura polygonata* on Yonagunijim Island was first noted in Okada's (1937) monograph on the basis of information from "KURODA". Curiously enough, however, Okada (1937), while introducing Van Denburgh's (1912) subspecific classification of *J. polygonata*, retained the Yonagunijima population in the nominotypical subspecies (p. 93). Nakamura and Uéno (1963) also assigned the Yonagunijima population to *J. p. polygonata*

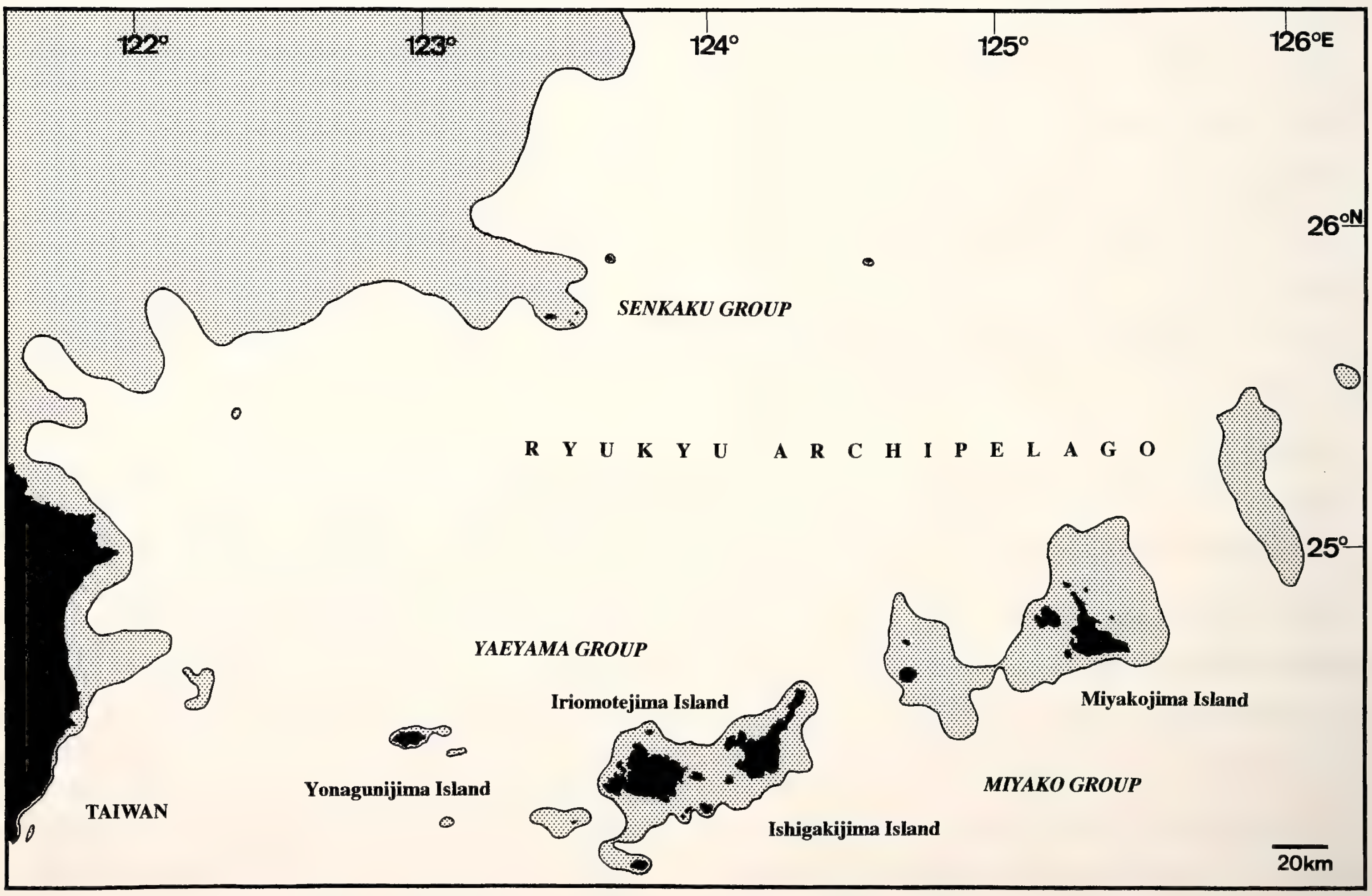

FIG. 1. Map of the southern Ryukyus and adjacent regions showing the location of Yonagunijima Island, the type locality of *Japalura polygonata donan* subsp. nov. The stippled portions denotes current sea areas that are considered to have constituted additional lands during the Late Pleistocene (i.e., sea areas shallower than 120 m: Ota et al., 1993).

together with all other Ryukyu populations under the assumption that *Japalura swinhonis* Günther, 1864 (including *J. mitsukurii* Stejneger, 1898: see Ota, 1991) of Taiwan should be regarded as a subspecies of *J. polygonata*, a view clearly negated on the basis of recent morphological and ecological observations by Ota (1988a, 1991). Matsumoto (1979), on the other hand, implicitly synonymized Van Denburgh's (1912) *J. p. miyakensis* with *J. p. ishigakiensis*, because he referred to all Yaeyama (including Yonagunijima Island) and Miyako populations as *J. p. ishigakiensis*.

Recently I had a chance to examine a good series of *Japalura* specimens from Yonagunijima Island for the first time. Comparison of these specimens with specimens of *J. polygonata* from many other islands of the Ryukyus and Taiwan revealed the presence of slight but consistent differences between the Yonagunijima specimens and the remainder. However, they exclusively shared an orange [males] or yellow [females] spot at the center of the gular. Because such a gular spot was not observed in other species so far studied (Ota, 1991), I tentatively regard this spot as synapomorphy of *J. polygonata*, and thus describe the Yonagunijima population as a new subspecies of this taxon.

Materials and Methods

A total of 56 specimens (42 adult males, 11 adult females, and three juveniles) collected from Yonagunijima Island were compared with 354 specimens of *J. p. polygonata* from eight islands of the central Ryukyus, 177 specimens of *J. p. ishigakiensis* from three islands of the southern Ryukyus, and 148 specimens of *J. p. xanthostoma* from four localities in northern Taiwan (see Appendix for further details). Of the 56 Yonagunijima specimens, two males and two females were also subjected to karyotyping following Ota (1988b).

Morphological characters were examined following Ota (1991). Sexual difference in adult snout-vent length (SVL) was examined by t-test, and was also expressed by the sexual dimorphism index (SDI) proposed by Gibbons and Lovich (1990) as:

$+x/y$, when $x>y$; or $-y/x$, when $x<y$

where x and y denote means for adult females and adult males, respectively. Differences in meristic characters among samples were tested for statistical significance by Kruskal-Wallis test and Dunn's multiple comparison test following Zar (1984).

In the following sections, catalogue numbers of specimens deposited in the Zoological Collection of the Kyoto University Museum, and Masami Hinoue's private collection are preceded by KUZ and HPN, respectively. The other institutional acronyms follow Leviton et al. (1985).

Japalura polygonata donan subsp. nov.

Fig. 2

Japarula [sic] *polygonata polygonata*: Okada, 1937:93 (part).

Japalura polygonata polygonata: Nakamura and Uéno, 1963:103 (part).

Japalura polygonata ishigakiensis: Matsumoto, 1979:17 (part); Ota, 1983:16 (part); Toyama, 1985:63 (part); Ikehara et al., 1994: 275 (part); Ota, 1991:288 (part).

Holotype

KUZ R234, an adult male collected at Mt. Urabudake (24°27'N, 123°00'E, alt. 130 m), Yonagunijima Island, on 17 August 1981 by H. Ota.

Paratypes

KUZ R102, an adult female collected at Higawa, Yonagunijima Island, on 30 March 1981 by H. Ota; KUZ R231, 238, two adult females, sampling data same as the holoyupe; KUZ R415, an adult female collected between Higawa and Arakawabana, Yonagunijima Island, on 7 March 1982 by H. Ota; KUZ R971, 972, 975, 979, 997, four adult males and one adult female collected between Mt Urabudake and Sonai, Yonagunijima Island, on 26 March 1983 by T. Hayashi, K. Kawamura, S. Kobayashi, A. Mori, and H.

FIG. 2. Male paratype (A. KUZ R3294) and female paratype (B. KUZ R4515) of *Japalura polygonata donan* subsp. nov.

Ota; KUZ R1382, 1420, two adult females collected at Kubura, Yonagunijima Island, on 27 July 1983 by H. Ota; KUZ R2000–2003, 2164, 2876, 3261–3263, 3265, 3269, 3270, 3281–3283, 3289, 3291–3294, 3982, 4515, 4517, 4518, 22 adult males, two adult females, and three juveniles collected at Mt. Urabudake on 15 July 1984 by H. Ota; KUZ R28117, 28118, two adult females collected at Kubura on 25 June 1993 by H. Ota; 56152–56156, 56158–56167, 15 adult males collected at Mt. Urabudake on 25 March 2003 by H. Ota.

Diagnosis

Adult females largest among the subspecies of *J. polygonata* (55.5–70.5 mm in snout-vent length [SVL] [$\bar{x}$=63.3]), brilliant green dorsally. Adult males almost as large as females (57.7–71.0 mm SVL [$\bar{x}$=65.6]), with a series of large white spots against dark grayish tan on the lateral surface of body. Middorsal scales 30–44 ($\bar{x}$=37.1), fewer than those in other subspecies, but with some range overlaps (34–47 [$\bar{x}$=40.8] in *J. p. polygonata*; 35–53 [$\bar{x}$=44.3] in *J. p. ishigakiensis*, and 37–48

[x̄=42.4] in *J. p. xanthostoma*); some dorsolateral body scales distinctly enlarged, irregularly arranged; orange (male) or yellow (female) spot at center of gular in adults; sexual size dimorphisms not evident; buccal and palatal mucosa light pinkish gray.

Etymology

The subspecific epithet, "*donan*", refers to an old vernacular name of Yonagunijima Island. "Donan" is not in official use at present. However, this term is still commonly used by the inhabitants of this island. "Donan" also refers to a kind of special liquor of Yonagunijima, which added a great joy to my fieldwork there.

Description of holotype

Measurements (in mm): SVL 69.4; head length 21.8; snout-eye length 9.0; interorbital distance 10.9; fore-limb length 33.6; axilla to groin distance 31.8; hindlimb length 55.4; tibia length 18.1; toe IV length 13.4; tail length 178.1.

Snout tapering, roundish at tip; rostral quadrangular, about three and a halh times as wide as long, covering anterior tip of snout; seven scales, in contact with rostral; nasal separated from rostral by one scale, contacted by this intervening scale, first supralabial, and six other scales; supralabials not keeled, seven on left, six on right, second separated from nasal by one scale; a longitudinal row of keeled and moderately enlarged scales, each larger than half the size of supralabials, behind nasal to imaginary vertical line from posterior margin of eye; one row of much smaller scales intervening between preceding row and supralabials; mental pentagonal, surrounded by four scales; infralabials slightly keeled, seven on both sides; a row of enlarged scales on anterior part of lateroventral corner of mandible, first ones on both sides and second one on right side contacting first infralabials, the others separated from labial series by one or two scales; tympanum completely concealed beneath skin; dorsal and lateral surfaces of head covered with enlarged and irregularly arranged scales, each with a distinct midline keel; some scales in occipital region with a few additional weak keels, making surface rugose; interparietal surrounded by eight scales; superciliaries imbricate, each overlapping one third to half with successor; scales around eye very small; scales on ventral surface of head obliquely arranged, most of them more or less keeled medially; but a few not keeled; longitudinal gular fold very slight; slender, hair-like sense organs present on several head scales; longitudinal middorsal scale row consisting of 40 enlarged, strongly keeled scales, anterior eight especially enlarged, and much compressed, each almost as broad as high, forming a nuchal crest; scales adjacent to middorsal row smaller, directed backward and slightly upward; most scales in dorsolateral and lateral regions of body weakly keeled, as large as or smaller than those on venter, intermixed with much enlarged, strongly keeled, irregularly arranged scales; scales on ventral surfaces of body and limbs moderately keeled and regularly arranged longitudinally; slight oblique fold on each shoulder; limbs relatively short, toe IV (exlusive of claw) reaching to middle of eye when hindlimb adpressed forward; scales on dorsal surface of limb larger than those on ventral surface; finger and toe I shortest, finger IV almost as long as finger III, toe IV distincly longer than toe III; subdigital scales 9–9, 13–13, 18–19, 19–19, 12–12 on left-right fingers I, II, III, IV, Y, 8–7, 13–13, 20–20, 23–25, 16–16 on left-right toes I, II, III, IV, V; tail slightly compressed, oval in cross section, with slightly enlarged median scales on dorsum; subcaudal scales strongly keeled, distinctly larger than scales on dorsum.

Color in life

Dorsal ground color dark grayish tan; infraorbital region paler than surroundings but not white; otherwise, no distinct markings on head; each side of body with five quadrangular white spots forming a longitudinal row; second and third spots partially fused on left side, separated on right side; ground color of limbs and tail slightly lighter than that of trunk,

several indistinct dark annuli on limbs, 15 dark annuli on tail.

Ventral surface of head light gray, without markings except for one large orange spot in midst of gular region; ventral surfaces of remaining part of body pale gray. Buccal-palatal mucosa light pinkish gray.

Color in ethanol

Dorsal ground color turned slightly paler; the orange spot on gular faded to invisible; the remaining color patterns similar to those in life.

Variation

Variations in some meristic characters and SVL are given in Tables 1 and 2, respectively. There are no significant differences in those meristic characters between the sexes (P>0.05). Unlike in *J. p. polygonata* and *J. p. ishigakiensis* where sexual size dimorphism is evident with males being larger, *J. p. donan*, as well as *J. p. xanthostoma*, does not show significant differences in adult SVL between sexes (Table 2).

Live coloration of females remarkably differs from that of males. Dorsal surface of

TABLE 1. Variation in meristic characters ($\bar{x}\pm SD$, followed by ranges in parentheses) among island samples of *Japalura polygonata* examined. Abbreviations are: SER, number of scales contacting rostral; IOS, number of scales touching an imaginary line drawn between outer edges of left and right supraciliaries; DC, middorsal crest-like scales from just posterior to occipital granules to just above anterior margin of vent; T4S, slightly enlarged scales beneath toe IV, counted from branching point with toe III to base of claw.

Subsp.	Island	n	SER	IOS	DC	T4S
polygonata	Kikaijima	34	8.31±0.82 (7–9)	12.46±1.28 (10–15)	39.77±2.12 (36–44)	25.54±1.28 (23–27)
	Amamioshima	17	8.61±0.70 (7–10)	12.87±1.10 (11–15)	41.23±2.50 (37–45)	25.55±1.27 (23–28)
	Kakeromajima	33	8.55±0.74 (7–10)	12.58±1.16 (11–15)	40.12±2.53 (36–45)	25.76±1.32 (23–28)
	Tokunoshima	53	8.52±0.78 (7–10)	12.26±1,30 (10–15)	41.32±2.78 (35–45)	25.52±1,18 (23–27)
	Iheyajima	28	8.65±0.70 (7–10)	12.02±0.99 (11–15)	41.16±2.42 (36–44)	25.30±1.24 (23–27)
	Okinawajima	61	8.29±0.98 (6–10)	11.95±1.16 (10–14)	40.72±3.06 (33–49)	26.05±1.31 (23–29)
	Tokashikijima	63	8.03±0.85 (7–9)	12.13±1.17 (9–14)	39.44±2.40 (35–43)	25.50±1.68 (23–30)
	Kumejima	65	8.25±0.82 (6–10)	12.04±1.22 (10–15)	40.04±2.84 (34–44)	25.84±1.47 (23–28)
ishigakiensis	Miyakojima	41	8.01±1.02 (5–11)	13.22±1.19 (11–16)	44.80±3.27 (39–52)	23.52±1.23 (21–26)
	Ishigakijima	80	8.40±0.87 (6–10)	12.41±1.17 (11–15)	45.62±2.51 (39–52)	22.99±1.50 (20–27)
	Iriomotejima	56	7.78±1.08 (6–10)	12.91±0.97 (10–15))	44.04±2.85 (37–52)	23.25±1.40 (19–26)
donan	Yonagunijima	53	7.31±0.56 (7–9)	11.74±1.16 (10–14)	37.17±2.68 (30–44)	22.82±1.23 (20–27)
xanthostoma	Taiwan	148	8.06±0.94 (5–10)	13.15±0.90 (11–15)	44.39±3.43 (38–55)	23.55±1.59 (20–29)

TABLE 2. Variation in adult male and female SVLs (mm) in island samples of *Japalura polygonata* examined. Significance levels of difference in adult SVL between sexes from the same islands are as follows: +++, P<0.001; ++, P<0.01; –, P>0.05. SDI, sexual dimorphism index (Gibbons and Lovich, 1990: see text).

Subsp.	Island	Sex	n	x̄	SD	Range	SDI
polygonata	Kikaijima+++	male	27	67.6	5.8	55.1–79.2	–1.164
		female	7	58.1	5.2	51.5–68.8	
	Amamioshima++	male	7	68.9	6.1	54.0–80.0	–1.164
		female	10	59.2	5.0	51.0–69.5	
	Kakeromajima+++	male	21	68.0	6.0	54.3–79.0	–1.174
		female	11	57.9	4.8	51.8–69.0	
	Tokunoshima +++	male	42	69.1	6.3	54.2–80.5	–1.161
		female	11	59.5	5.4	53.9–69.2	
	Iheyajima+++	male	18	68.1	5.8	53.0–77.6	–1.178
		female	10	57.8	4.3	50.8–68.2	
	Okinawajima+++	male	55	67.1	6.6	54.0–80.2	–1.128
		female	33	59.5	3.9	52.5–68.5	
	Tokashikijima+++	male	34	67.3	5.4	53.8–76.9	–1.152
		female	28	58.4	4.1	53.1–67.9	
	Kumejima+++	male	40	67.4	6.2	54.2–80.0	–1.164
		female	22	57.9	5.1	52.1–68.2	
ishigakiensis	Miyakojima++	male	22	62.2	6.9	47.5–72.0	–1.150
		female	12	54.1	3.6	51.0–62.0	
	Ishigakijima+++	male	56	59.9	3.2	52.9–66.0	–1.093
		female	24	54.8	3.8	48.0–61.0	
	Iriomotejima++	male	37	59.4	3.7	48.5–67.0	–1.094
		female	15	54.3	3.5	47.0–60.5	
donan	Yonagunijima–	male	42	65.6	2.7	57.7–71.0	–1.036
		female	11	63.3	4.4	55.5–70.5	
xanthostoma	Taiwan–	male	112	60.8	2.9	50.3–68.0	–1.020
		female	37	59.6	3.2	51.7–65.9	

body of females is brilliant green, with no white spots but with several obscure dark cross bands around the middorsal region. In females, there is a somewhat obscure yellow spot in midst of gular region.

Karyotype

Like other subspecies of *J. polygonata* (Ota, 1991), *J. p. donan* has a karyotype consisting of 2N=46 uniarmed chromosomes in a graded series.

Distribution and habitat

Japalura polygonata donan is endemic to Yonagunijima Island of the Yaeyama Group, Ryukyu Archipelago, Japan. Most specimens were found in the marginal portions of well-recovered secondary forests consisting of evergreen broad-leaf trees.

Ecological notes

All three females collected in March had ovarian follicles (3–5 mm in diameter) only, whereas the six females collected between mid July and mid August exhibited flaccid, empty

oviducts and poorly developed ovarian follicles (<3 mm in diameter). Of the remaining two females, both collected in the late June, one had two and one large follicles (7 mm in diameter) in left and right ovaries, respectively. The other had one elliptic egg (15.8×7.5 mm) in each oviduct. It is thus probable that in this subspecies ovulation and oviposition occur chiefly in late June or early July.

One colubrid snake, *Dinodon rufozonatum walli*, collected at Mt. Urabudake in August 1981, had two slightly digested adult male *J. p. donan* in its stomach.

Discussion

Japalura polygonata donan shares an orange (males) or yellow (females) spot in the center of the gular exclusively with the other conspecific subspecies. However, it differs from the latter in having distinctly enlarged and irregularly arranged scales on the dorsolateral surface of the body. In other subspecies, the degree of enlargement of such scales is smaller, and they usually form somewhat regular rows in a transverse direction on the flanks, and in a longitudinal direction in the paravertebral region. In the present subspecies, the middorsal scales, ranging from 30 to 44 ($\bar{x}$=37.1), are fewer than in other subspecies, although there are some range overlaps (34–47 [$\bar{x}$=40.8] in *J. p. polygonata*; 35–53 [$\bar{x}$=44.3] in *J. p. ishigakiensis*, and 37–48 [$\bar{x}$=42.4] in *J. p. xanthostoma*: Tables 1 and 3). The males of *J. p. donan* differs from the males of other subspecies in having a series of large white spots against dark grayish tan on the dorsolateral surface of the body. In other subspecies, the dorsolateral surface of the body has a continuous white or yellow band instead. The adult females of *J. p. donan* are the largest among the females of *J. polygonata* populations (55.5–70.5 mm in SVL, [$\bar{x}$= 63.3 mm]), and are almost as large as the adult males of the same subspecies (57.7–71.0 mm SVL [$\bar{x}$=65.6]). Thus, the male large sexual size dimorphism is not evident in this subspecies unlike in other subspecies exclusive of *J. s. xanthostoma* (Table 2). The females *J. p. donan* are also characterized by the brilliant green coloration on the dorsum of the body. The buccal and palatal mucosa of the present subspecies is not yellowish as in *J. p. xanthostoma*.

Taxonomic treatment of allopatric, slightly but diagnosablely diverged entities like the Yonagunijima population of *J. polygonata* is a matter of serious debate among taxonomists, depending on which school they belong to (Frost and Hillis, 1990; Grismer et al., 1994). In the present case, the population is clearly diagnosable (see above). Moreover, from the geographic pattern of variation in *J. polygonata* as a whole (Table 3), naming of the Yonagunijima population is not an arbitrary slicing of a geographically clinal entity, a

TABLE 3. Comparisons of island samples of *Japalura polygonata* by Kruskal-Wallis test (KWT) and Dunn's multiple comparison test. Abbreviations of island samples are: KK, Kikaijima; AO, Amamioshima; KR, Kakeromajima; TN, Tokunoshima; IH, Iheyajima, OK, Okinawajima; TK, Tokashikijima; KM, Kumejima; MY, Miyakojima; IS, Ishigakijima; IR, Iriomotejima; YN, Yonagunijima; TW, Taiwan. See Table 1 for character abbreviations. Significance levels for KWT are: +++, P<0.001; +, P<0.05. Samples sharing same superscript letters showed no significant differences in Dunn's multiple comparison test (P>0.05).

Characters	KWT	Descending order of samples												
SER	+++	IH^{a}	AO^{a}	KR^{ab}	TN^{ab}	IS^{bc}	KK^{bc}	OK^{c}	KM^{c}	TW^{d}	TK^{d}	MY^{de}	IR^{e}	YN^{e}
IOS	+	MY^{a}	TW^{a}	IR^{ab}	AO^{bc}	KR^{abc}	KK^{bc}	IS^{bcd}	TN^{bcd}	TK^{cd}	KM^{cd}	IH^{cd}	OK^{de}	YN^{e}
DC	+++	IS^{a}	MY^{ab}	TW^{ab}	IR^{b}	TN^{c}	AO^{c}	IH^{cd}	OK^{cd}	KR^{cd}	KM^{d}	KK^{d}	TK^{d}	YN^{e}
T4S	+++	OK^{a}	KM^{a}	KR^{a}	AO^{a}	KK^{a}	TN^{a}	TK^{a}	IH^{a}	TW^{b}	MY^{bc}	IR^{bcd}	IS^{cd}	YN^{d}

practice that can not be justified by the standards of modern taxonomy (e.g., Frost and Hillis, 1990). Also, one may argue that the Yonagunijima population deserves the status of a full species rather than a subspecies of *J. polygonata*. However, the presence of less diverged populations on both sides of Yonagunijima Island that are currently treated as two conspecific subspecies (i.e., *J. p. ishigakiensis* of the remaining Yaeyama Group and Miyako Group islands, and *J. p. xanthostoma* from Taiwan: Ota, 1991) makes it safer at present to retain the Yonagunijima population also at the subspecific status. Future detailed investigations of historical relationships at the population level and captive hybridization experiments may lead to elevation of *J. p. donan* to the full species status.

Yonagunijima Island is separated from other neighboring islands and the continent by straits deeper than 500 m (Maritime Safety Agency, 1978: Fig. 1). This suggests that the island has been isolated for a long period of time (e.g., Ota et al., 1993). *Japalura polygonata donan* and the two other reptiles endemic to Yonagunijima Island (i.e., *Calamaria pavimentata miyarai* and *Elaphe carinata yonaguniensis*) are likely to have diverged from their relatives in Taiwan and the southern Ryukyus through such an isolation.

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APPENDIX

Comparative specimens examined

Japalura polygonata polygonata. Ryukyu Archipelago: Okinawa Group: Kerama Islands (type loc.): Tokashikijima Is.: KUZ R2300–2306, 2360–2369, 4082, 4085, 4087–4101, 4494–4514, 4568, 4569, 4574–4576, OPM H0187, 0188, 0437; Okinawa Group, Kumejima Is.: KUZ R2241, 2333, 2334, 2421–2424, 4077, 4444–4493, 4578–4582, OPM H0082, 0451; Okinawa Group: Okinawajima Island: AMNH 21167–21169, 21172, HPN 42, 51, 55, 56, 59, 60, 69–71, 88–91, 383, 439, KUZ R1146–1200, 1355–1358, 1380, 1381, 1383–1385, 1423, 1483, 1485, OMNH R93–98, 733, 734, 736, 737, 744, 759–762, 897, 898, 1393, 1394, 1451–1453, 1455–1458, 1460, 1618, 1704); Okinawa Group: Iheyajima Is.: KUZ R2267, 2307; 2308, 2370–2378, 3950–3958, 4083, 4086; 4570–4573, OPM HJ0029; Ryukyu Archipelago: Amami Group: Tokunoshima Is.: KUZ R2159–2162, 2425–2429, 2940, 2943–2945, 3000, 3014, 3057, 3102, 3224, 3225, 3286, 3457, 3465, 3965, 4003–4031, 4033; Amami Group: Kakeromajima Is.: KUZ R2396–2408, 3984–3999, 4063–4066; Amami Group: Amaioshima Is.: KUZ R2163, 2379–2389, 2968–2971, OPM H0028; Amami Group: Kikaijima Is.: KUZ R2266, 2321, 2335, 2337, 2338, 2393–2395, 2409–2420, 3962, 4059–4062, 4067–4076.

J. p. ishigakiensis. Ryukyu Archipelago: Yaeyama Group: Ishigakijima Is. (type loc.): KUZ R3–5, 225, 227, 702–714, 719–722, 903, 905, 906, 934–936, 1023, 1373–1379, 1386, 1474–1480, 2008, 2205–2207, 2218, 2219, 2222–2224, 2226, 2877, 2878, 2880–2883, 2885, 2897, 2928, 3052, 3279, 3280, 3290, 3977, 4519. OMNH R772, 786, 787, 1653, 1696–1699, OPM H0054, 0093; Yeyama Group: Iriomotejima Is.: KUZ R2196–2204, 2356–2359, 2390–2392, 3274, 3275, 3947, 3948, 3959–3961, 3978, 3979, 4102, 4103, 4365, 4366, 4521, 4522, 4524–4537, 4540, OPM H0025, 0051, 0052, 0074, 0081, 0085, 0183–0186; Ryukyu Archipelago: Miyako Group: Miyakojima Is. (tyoe loc. of *J. p. miyakensis*: KUZ R2150–2158, 2183, 2339–2348, 4080, 20 uncatalogued specimens.

J. p. xanthostoma. Taiwan: Taipei: Waishuangchi (type loc.): KUZ R9855 (holotype), KUZ R1600–1602, 1609, 1610, 1649, 1657, 2058, 2059, 2715, 2809, 2919–2923, 2933, 2978, 2979, 3036, 3056, 3219, 3964, 4360, 4361, 6707–6710, 7519–7534, 7537–7545, 7846, 7851–7855, 7861–7871, 7880–7882, 7884, 7901, 7922, 7941, 7942, 7952, 7961, 7973, 7991, 7992, 8009, 8010, 8019, 8020, 8066, 8067, 8099, 8420, 8446–8452, 9775–9780, 9842–9854, 9856–9861, 13010–13019; Taipei: Tansui: KUZ R8424–8431; Taipei: Peitou: AMNH 77093; Taiwan: Ilan: Tungao: KUZ R6914, 6915, 6943, 6944, 6947(all paratypes).

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Normal Development of *Microhyla ornata*: The First Description of the Complete Embryonic and Larval Stages for the Microhylid Frogs (Amphibia: Anura)

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Abstract: Developmental changes in external morphological characters were examined for the whole embryonic-larval period of *Microhyla ornata* from Okinawajima Island, Ryukyu Archipelago, Japan. By considering the extent of morphological changes observed and compatibility with standard developmental tables hitherto proposed for other anuran groups, a developmental table consisting of 45 developmental stages was proposed for this species. Eight developmental terms, each consisting of several successive stages, were also defined—Cleavage-Blastula [stages 1–10], Gastrula [stages 11–14], Neurula [stages 15–18], Tail bud [stages 19–21], External gill [stages 22–28], Hind limb bud [stages 29–33], Hind limb formation [stages 34–41], and Metamorphosis [stages 42–45]). From previously described conspecific samples from other localities, our sample exhibited appreciable differences in the egg diameter, timing of pigmentaion at the position of the stomodium, and a few other tadpole characters.

Key words: Developmental table; External characters; *Microhyla ornata*; Microhylidae; Anuran amphibians; Okinawajima Island

INTRODUCTION

One of the prominent life history characteristics common to most living amphibians is the presence of an aquatic larval period, which immediately follows the initial embryonic development after fertilization and ends with the completion of metamorphosis (Duellman and Trueb, 1994; Altig and McDiarmid, 1999a). During the larval period, amphibians, anurans in particular, exhibit a series of dramatic morphological changes (e.g., tail formation, perforation and closure of the spiracle, limb formation, tail reduction). Appropriate staging of the larval period is, therefore, fundamental to various life history studies of amphibians. Indeed, tables of developmental stages that describe morphological changes during the larval period have been proposed for quite a few amphibian taxa (see Gosner, [1960], Nieuwkoop and Faber [1967], Fox [1983], Iwasawa and Futagami [1992], McDiarmid and Altig [1999], and other works cited therein). Nevertheless, such developmental tables are not yet available at all for several major groups of anurans.

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The family Microhylidae is a well defined monophyletic group of anurans (Ford and Cannatella, 1993; Haas, 2003), and is composed of more than 60 genera and 310 species from tropical, subtropical, and partially temperate regions of Asia, northern Australia, sub-Saharan Africa (including Madagascar), and the New World (Frost, 1985; Duellman, 1993). Because of the presence of several prominent morphological and ecological features (Altig and Johnston, 1989; Altig and McDiarmid, 1999a, b; Haas, 2003), tadpoles of the Microhylidae have been a subject of intensive evolutionary, systematic, and anatomical studies (e.g., Starrett, 1973; Wassersug, 1980, 1984, 1989; Wassersug and Pyburn, 1987; Khan, 2000). Nevertheless, complete tables of normal larval development are not yet available for any member of this family (Fox, 1983; McDiarmid and Altig, 1999).

Therefore, we have prepared a developmental table, covering all embryonic and larval periods, for *Microhyla ornata*, an Oriental microhylid species broadly distributed from South and Southeast Asia to southern China and the Ryukyu Archipelago (Frost, 1985; Zhao and Adler, 1993: but see Kuramoto [1976, 1987], Dubois [1987], Maeda and Matsui [1989], and Khan [2000] for the possibility of its being a composite of more than one species). For this species, a few previous studies yielded data on some aspects of reproduction and life history, such as those on the reproductive season (Kuramoto, 1973; Schleich and Kästle, 2002), fecundity parameters (Matsui and Ota, 1984; Schleich and Kästle, 2002), dietary effect on larval growth (Dash and Dei, 1998), larval morphology at certain developmental stages (Chou and Lin, 1997; Khan, 2000; Schleich and Kästle, 2002; etc.), and size at metamorphosis (Maeda and Matsui, 1989; Dash and Dei, 1998). With respect to its embryonic and larval development, Liu et al. (1996) provided some descriptions on the basis of materials from continental China. These descriptions were, however, very brief and were limited to the period from fertilization to spiracle completion. Moreover, drawings depicting morphological features of the embryonic and larval stages given therein suffered extensive simplification and thus were not very informative.

Materials and Methods

Three pairs of *Microhyla ornata* in amplexus were collected from Kita-nakagusuku Village on Okinawajima Island in June 2003. These pairs were brought back to our laboratory (also located on Okinawajima), where they were separately housed in a plastic container (20×35 cm in area, 26 cm in height) filled with 10 l of tap water (25±1 C, left for a few days in advance). Eggs deposited were also kept in the same container under the same conditions until they had grown to larvae with complete spiracles. Then they were divided into several groups, each consisting of 100 or fewer individuals. Each group was housed separately in a container of the same size and amount of water as above. All containers with larvae were left outside in shade so as to let the larvae grow under natural temperature.

For each container, 2 of water was removed and the same amount of tap water (prepared and left for a few days in advance) was added every second day. A mixture of minced and dried shrimps and vegetables, sold as diet for aquarium fish, was used to feed the tadpoles. One gram of the mixture, further milled into powder, was scattered over the surface of water in each container after every change of water. Water temperature was measured to nearest 0.1 C with a digital thermometer between 16:00 and 17:00 every day.

Embryonic and larval developmental stages were defined so as to be as compatible as possible with those proposed by previous authors (Gosner [1961] for anurans in general, and Iwasawa and Futagami [1992] for *Hyla japonica*). For the description of each of the stages from fertilization (stage 1) to the completion of the spiracle (stage 27), five developing eggs or embryos were sampled at random. They were measured for the total length, fixed in 8% formalin, and subjected to

detailed observations under a dissecting microscope. For each of the stages subsequent to stage 27, at least five larvae were also randomly sampled. Each larva was put in a shallow dish filled with water, cooled until motionless by adding a piece of ice to the water, measured for the total length (TOL), body length (BL) and tail length (TAL), and subjected to detailed observations.

All measurements were taken to nearest 0.01 mm by a micrometer attached to a dissecting microscope. Drawings were prepared using camera-lucida.

Results

One of the male-female pairs deposited all eggs at one time after being housed in a container, whereas each of the remaining two pairs in two times. In the latter case, the second oviposition commenced approximately ten minutes after the initial oviposition. The total number of eggs laid by the three pairs was 220, 386, and 910. At deposition, the eggs formed a single layer on the water surface like many other species of the Microhylidae (Haas, 2003). From fertilization to the completion of metamorphosis took 40 days.

To document the series of changes in external morphological characters during the embryonic and larval development, a total of 45 developmental stages were defined (Table 1, Fig. 1). Following Iwasawa and Futagami (1992), these stages were grouped into eight terms as follows: Cleavage-blastula, consisting of stages 1–10; Gastrula, stages 11–14; Neurula, stages 15–18; Tail bud, stages 19–21; External gill stages 22–28; Hind limb bud,

TABLE 1. Stages of normal development of *Microhyla ornata*. Stages 1–28 were obtained during development at 25±1 C; Stages 29–45 at 19–26 C (natural outdoor temperatures of Okinawajima Island). Numerals with or without lower case letters in parentheses and brackets following stage numbers indicate numbers of grossly corresponding stages of Gosner (1960) and Iwasawa and Futagami (1992), respectively.

Stage number	Stage	Age	Diagnostic features (total length [in mm] is given as x±SD, followed by ranges and sample sizes in parentheses)
1 (1, 2) [1]	Early uncleaved	0:15 (h:m)	0.97±0.04 (0.93–1.04, n=5); rotation for orientation completed with upper location of animal hemisphere
2 (2) [1]	Late uncleaved	0:40	0.96±0.08 (0.83–1.07, n=5); upper part becoming flat, with gray crescent
3 (3) [2]	2-cell	0:53	1.01±0.11 (0.86–1.20, n=5); first cleavage (meridional), producing two blastomeres of equal sizes
4 (4) [3]	4-cell	1:06	0.95±0.04 (0.88–1.00, n=5); second cleavage (meridional), producing four blastomeres of equal sizes
5 (5) [4]	8-cell	1:19	1.00±0.07 (0.93–1.12, n=5); third cleavage (latitudinal), producing four small blastomeres above and four large blastomeres below
6 (6) [5]	16-cell	1:32	0.98±0.11 (0.83–1.16, n=5); fourth cleavage (meridional)
7 (7) [6]	32-cell	1:45	0.98±0.08 (0.85–1.08, n=5); fifth cleavages (latitudinal) more or less delayed in vegetal hemisphere

TABLE 1. —Extended.

8 (8a) [7a]	Morula	2:00	0.98±0.03 (0.96–1.04, n=5); sixth cleavages, occurring in animal hemisphere only
9 (8b) [7b]	Early blastula	3:00	0.95±0.08 (0.88–1.08, n=5); division of blastomeres continuing; each blastomere getting smaller
10 (9) [8, 9]	Late blastula	5:30	1.01±0.10 (0.88–1.16, n=5); cells in animal hemisphere minute, making surface smooth
11 (10a) [10]	Appearance of blastoporic lip	6:00	0.93±0.07 (0.85–1.04, n=5); invagination onset
12 (10b) [11a]	Horseshoe-shaped blastoporic lip	6:45	1.02±0.07 (0.93–1.12, n=5); blastoporic lip extending laterally, forming lateral lips
13 (11) [11b]	Large yolk plug	8:00	0.99±0.03 (0.96–1.04, n=5); lateral tips of blastoporic lip fusing ventrally, making blastopore circular; animal hemisphere starting to flatten
14 (12) [11c, 12]	Small yolk plug	9:30	1.00±0.11 (0.88–1.20, n=5); yolk plug becoming smaller; embryo slightly elongated along longitudinal axis
15 (13) [13]	Neural plate	10:30	1.26±0.04 (1.20–1.31, n=5); yolk plug invisible; primary neural fold distinct, secondary neural fold slight
16 (14) [14]	Neural fold	11:45	1.31±0.04 (1.24–1.36, n=5); secondary neural fold and neural groove distinct; dark pigmentaion at position of future stomodium
17 (15) [15]	Closure of neural fold	12:30	1.45±0.05 (1.36–1.52, n=5); neural folds contacting each other dorsomedially in whole trunk
18 (16) [16]	Neural tube	13:15	1.65±0.11 (1.47–1.75, n=5); neural tube completed; slight ridge at position of future stomodium; slight uplifting at position of future retina; tail bud small but visible
19 (17) [17]	Tail bud I	14:00	2.01±0.14 (1.83–2.23, n=5); tail bud elongated; stomodium and somatic structure in trunk musculature distinct; dark pigmentation at positions of future nares
20 (18a) [18a]	Tail bud II	15:00	2.46±0.24 (2.15–2.87, n=5); both dorsal and ventral portions of tail fin slightly developing; oral pit slight; with or without muscular response to mechanical stimulation
21 (18b) [18a]	Tail bud III	19:00	3.04±0.29 (2.55–3.43, n=5); tail fin distinct; cloacal tail piece distinct, medial; beginning to hatch

TABLE 1. —Extended.

22 (19, 20a) [19, 20a]	Appearance of gill bud	27:00	3.78±0.15 (3.59–3.99, n=5); primary external gill slightly protruding; positions at future nares slightly concave
23 (20b, 21a) [20b, 21a]	Elongation of external gills	32:00	3.83±0.20 (3.67–4.15, n=5); primary external gill extending, ramifying; secondary external gill protruding; mouth opening; cornea becoming transparent; heart beat and blood circulation visible from outside; starting to swim
24 (21b, 22) [21b, 22]	Completion of external gills	46:00	4.10±0.22 (3.67–4.31, n=5); primary external gill further elongated, reaching maximum length; secondary external gill ramifying; mouth becoming movable
25 (23) [23]	Opercular development I	52:00	4.20±0.18 (3.86–4.39, n=5); opercular folds appearing; epiderm starting to be transparent
26 (24) [24]	Opercular development II	59:00	4.29±0.34 (3.83–4.69, n=5); opercular folds covering external gills, but retainig small median opening
27 (25a) [25a]	Opercular development III	64:00	4.71±0.35 (4.31–5.26, n=5); spiracle opening at ventromedial position; mouth shifting to anterior tip of body; body becoming transparent
28 (25b) [25b]	Completion of spiracle	73:00	8.55±1.78 (4.70–11.32, 37); opening of spiracle shifting posteriorly; head and body extending laterally; mouth shifting to upper position; iridescent cells appearing in belly
29 (26) [26]	Appearance of limb bud I	7(d)	12.61±0.55 (12.05–13.52, 5); spur-like hind limb buds appearing; forelimb buds visible between gills and visceral organs; stomodium degenerating, leaving a patch of pigmentation
30 (27) [27]	Appearance of limb bud II	9	13.21±0.66 (12.05–14.26, 14); length of hind limb= 0.5×basal width
31 (28) [28]	Appearance of limb bud III	11	15.70±1.49 (13.38–17.64, 11); length of hind limb= basal width
32 (29, 30) [29, 30]	Appearance of knee junction	13	17.63±1.10 (15.44–19.11, 18); length of hind limb= 1.5×basal width; knee junction evident; patch of pigmentation at position of stomodium disappearing
33 (31) [31]	Oar-shaped limb bud	15	18.66±1.33 (16.17–20.87, 23); length of hind limb= 2×basal width; terminal portion of hind limb oar-shaped
34 (32, 33) [32, 33]	Appearance of 4th and 5th toes	17	21.09±1.31 (18.52–23.37, 25); shallow indentations appearing in margin of foot paddle between 3rd and 4th toes, and between 4th and 5th toes

TABLE 1. —Extended.

35 (34) [34]	Appearance of 3rd toe	19	21.99±1.00 (19.70–22.79, 8); shallow indentations appearing in margins of foot paddle between 2nd and 3rd toes, and in margins of hand paddle between 2nd and 3rd fingers and between 3rd and 4th fingers
36 (35, 36) [35, 36]	Appearance of 1st and 2nd toes	20	24.21±1.11 (22.49–26.46, 22); shallow indentations appearing in margins of foot paddle between 1st and 2nd toes, and in margins of hand paddle between 1st and 2nd fingers; femur, tibia, and foot distinct from each other
37 (37, 38a) [37, 38a]	Growth of hind limb I	22	25.46±1.36 (23.23–28.22, 23); 3rd, 4th, and 5th toes elongated, webbed; fingers also elongated
38 (38b) [38b]	Growth of hind limb II	25	25.80±0.95 (24.55–27.05, 12); 1st and 2nd toes elongated, webbed
39 (38c) [38c]	Appearance of metatarsal tubercle	29	26.45±1.74 (23.08–28.96, 12); metatarsal tubercle appearing; distal end of metatarsal separated from tail
40 (39, 40) [39, 40]	Appearance of tubercles below digital joints	31	26.81±1.98 (23.67–28.81, 10); tubercles developing on toes just beneath joints; metatarsal at right angle to tail; nares evident externally
41 (41) [41]	Involution of cloacal tail piece	35	25.64±1.91 (23.23–28.37, 15); cloacal tail piece commencing involutive change at base; tubercles beneath joints on toes distinct; slight transverse bands on hind limb; 3rd finger of left forelimb still covered by transparent pectoral skin, reaching above heart
42 (42, 43a) [42, 43a]	Degeneration of tail I	37	21.76±3.08 (16.02–23.81, 6); forelimbs emerging; tail fin and cloacal tail piece disappearing; tail rapidly diminishing
43 (43b, 44) [43b, 44]	Degeneration of tail II	38	15.91±3.94 (11.91–22.20, 5); tail further diminishing; head narrowing; mouth widening, shifting to anterior tip of head; slits of pectoral skin for emergence of forelimbs closing, remaining folds; toe webs starting to degenerate; eyeballs starting to protrude; spiracle disappearing; emergence
44 (45) [45]	Degeneration of tail III	39	8.32±1.12 (7.35–10.14, 5); tail stub-like; lateral ends of mouth reaching beneath anterior margin of eye
45 (46) [46]	Completion of metamorphosis	40	7.23±0.48 (6.32–7.94, 13); tail degeneration completing, making cloaca visible from above; lateral ends of mouth reaching beneath posterior margin of eye; pectoral folds almost gone

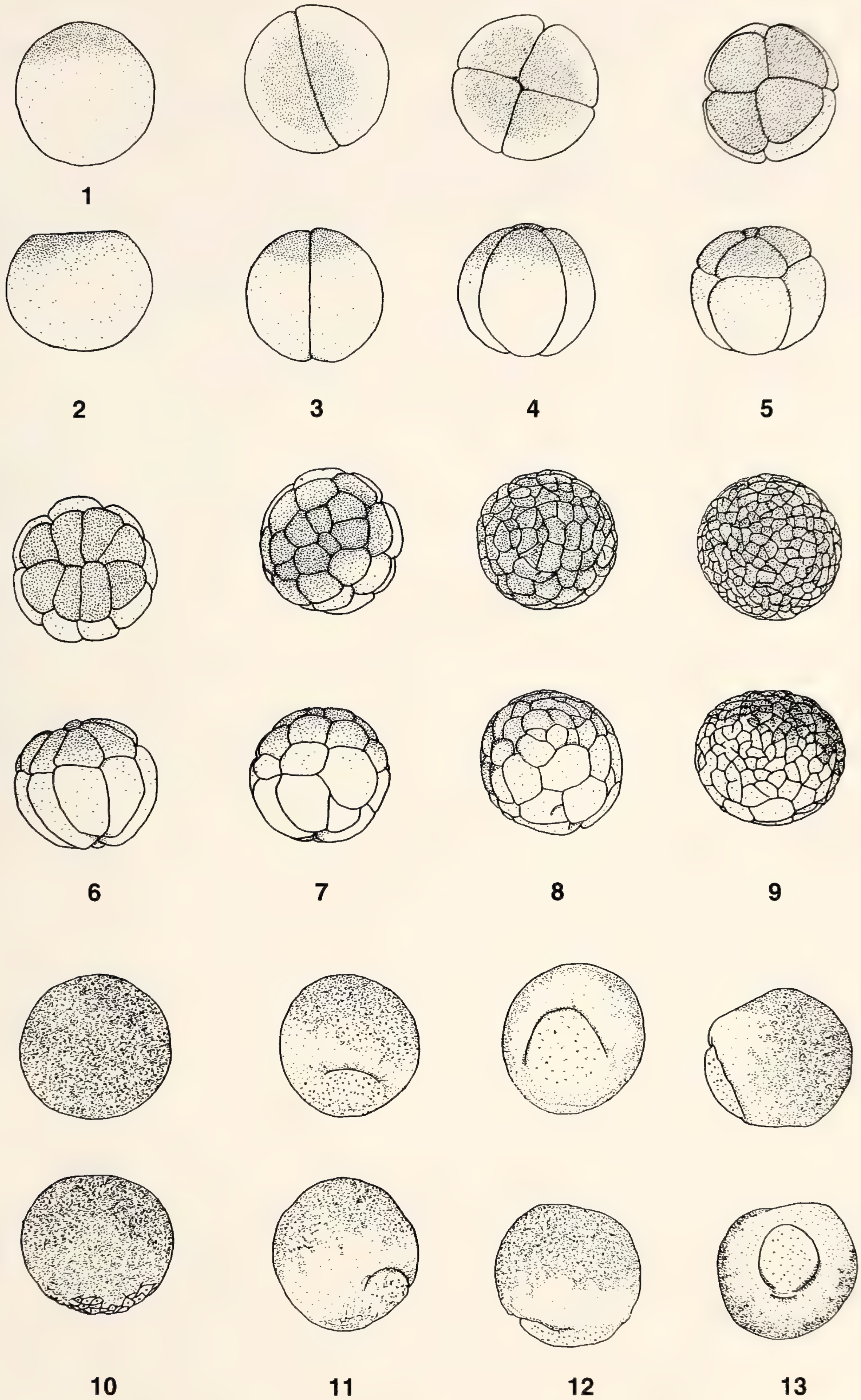


FIG. 1. Sketches of embryos (including developing eggs) and larvae of *Microhyla ornata* from Okinawajima. Numeral beneath each drawing or each group of drawings corresponds to that of the developmental stage defined in Table 1.

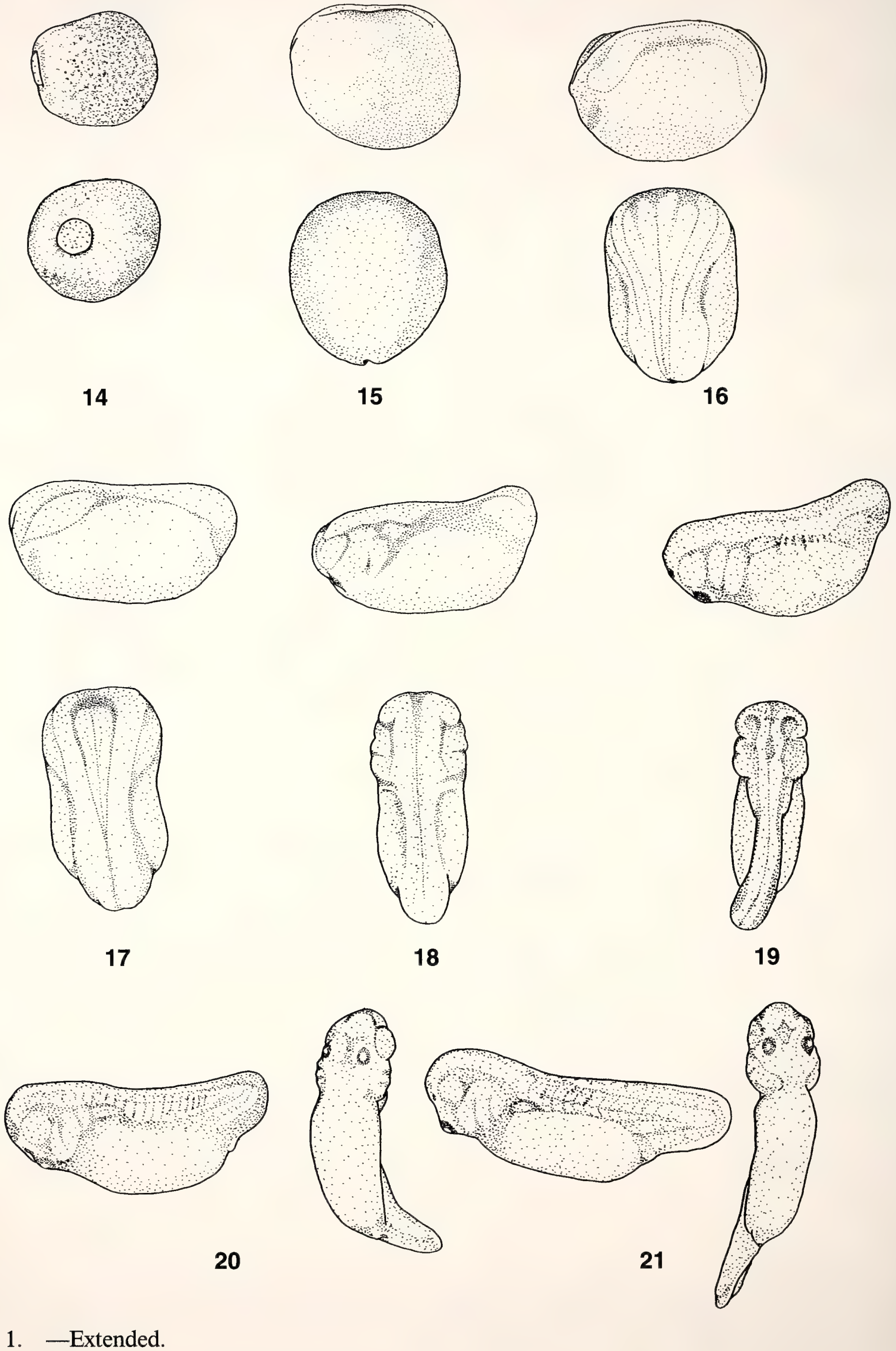


FIG. 1. —Extended.

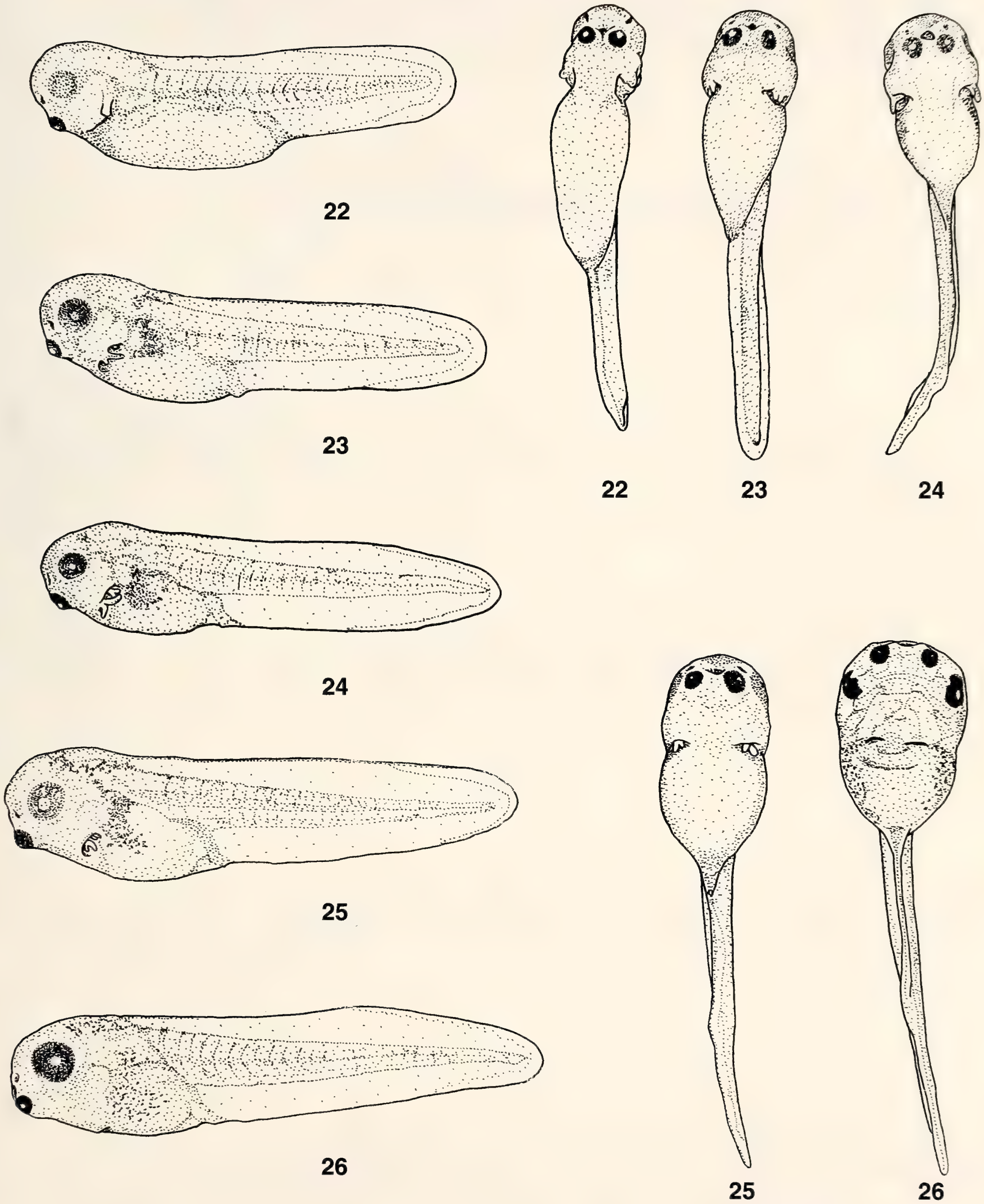


FIG. 1. —Extended.

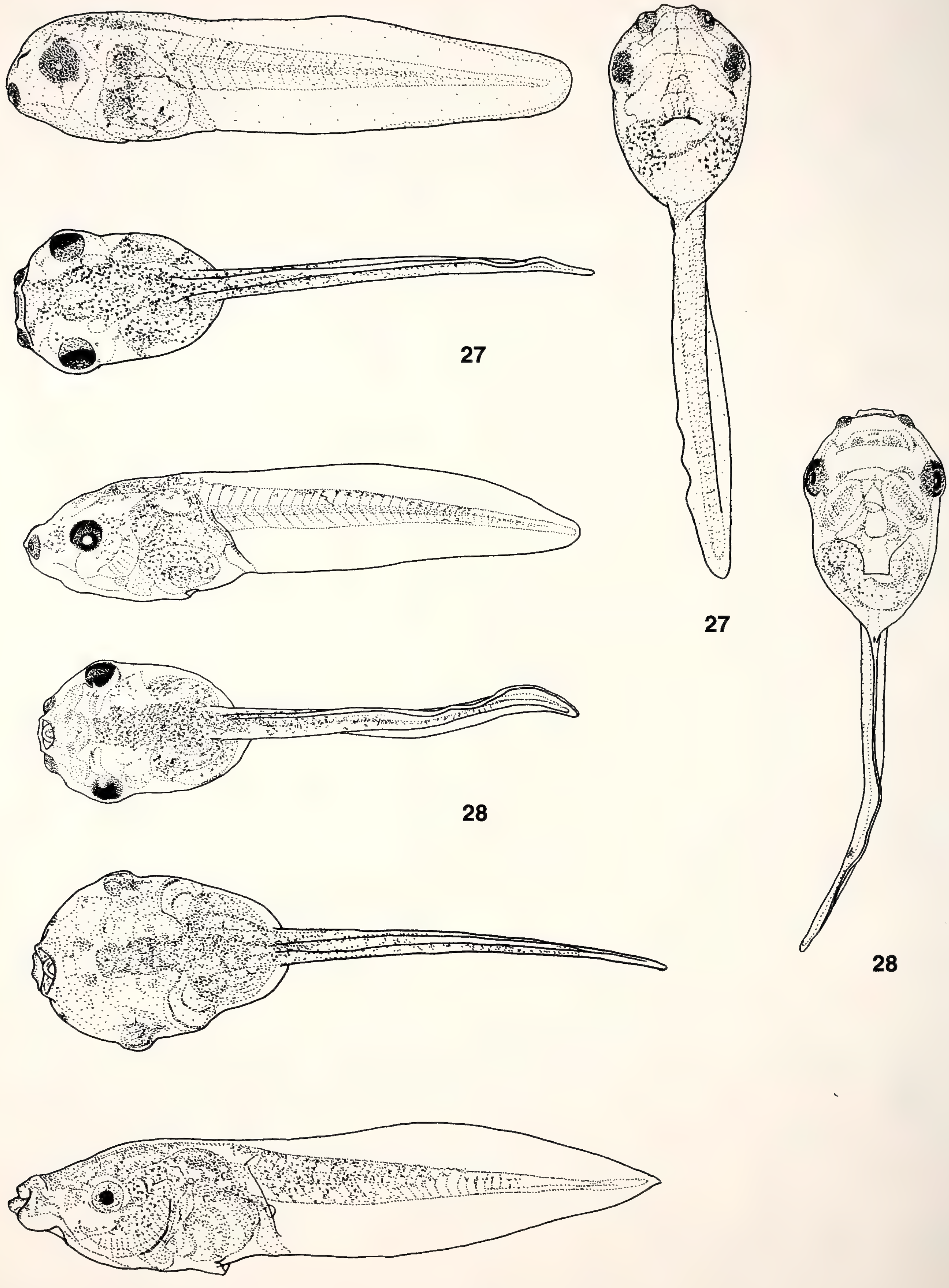
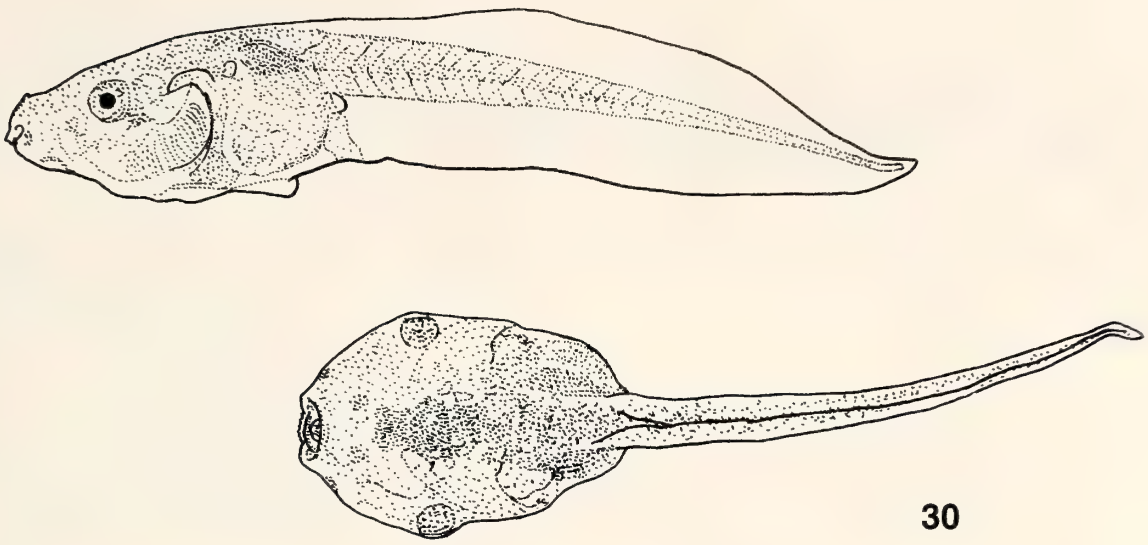


FIG. 1. —Extended.



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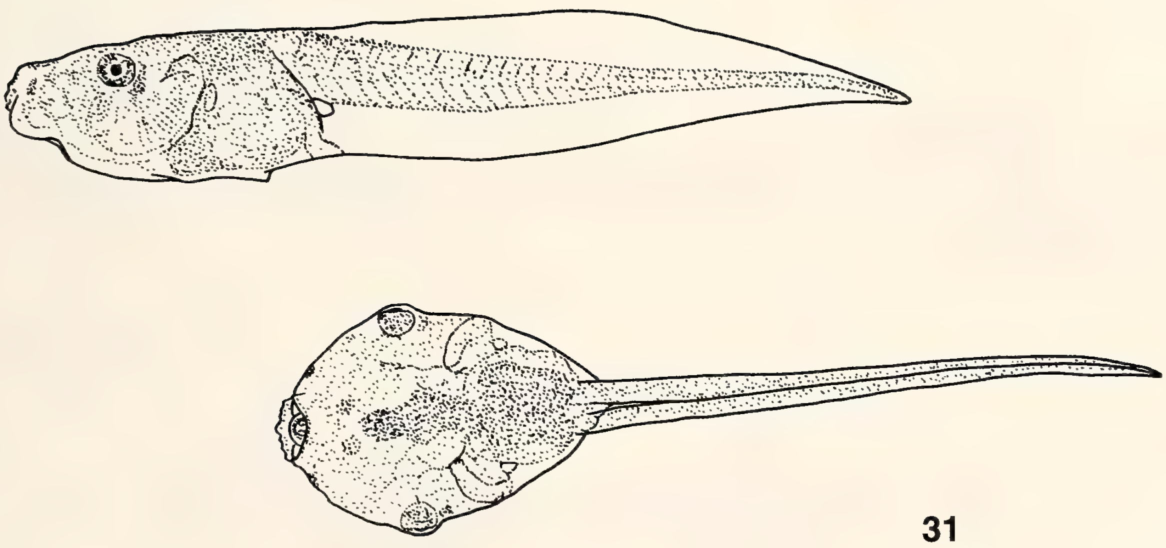

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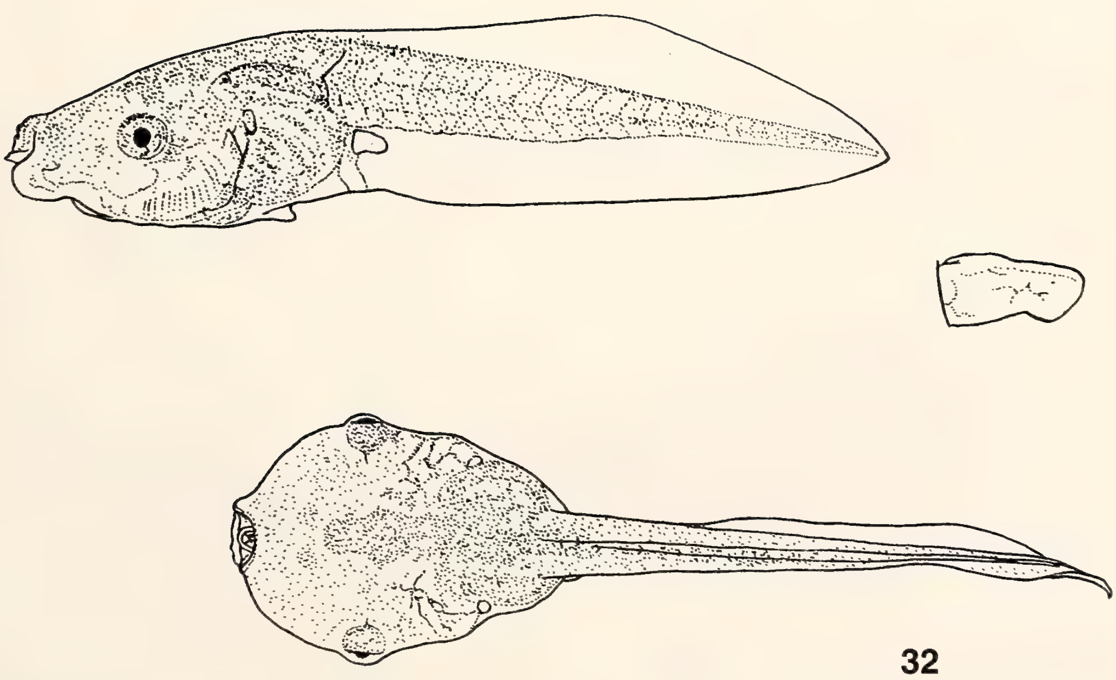

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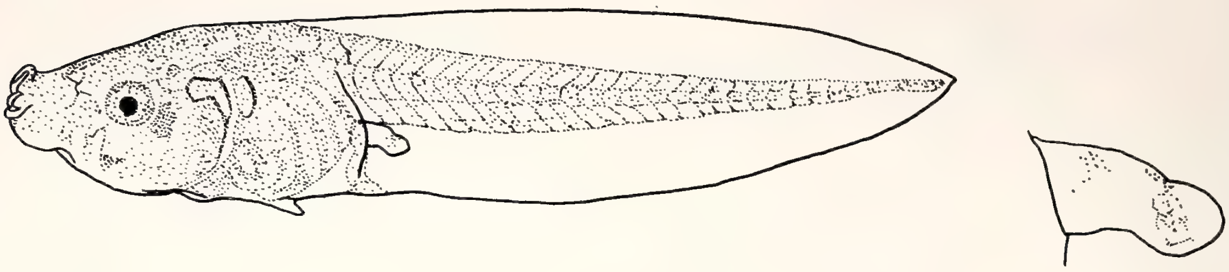
FIG. 1. —Extended.



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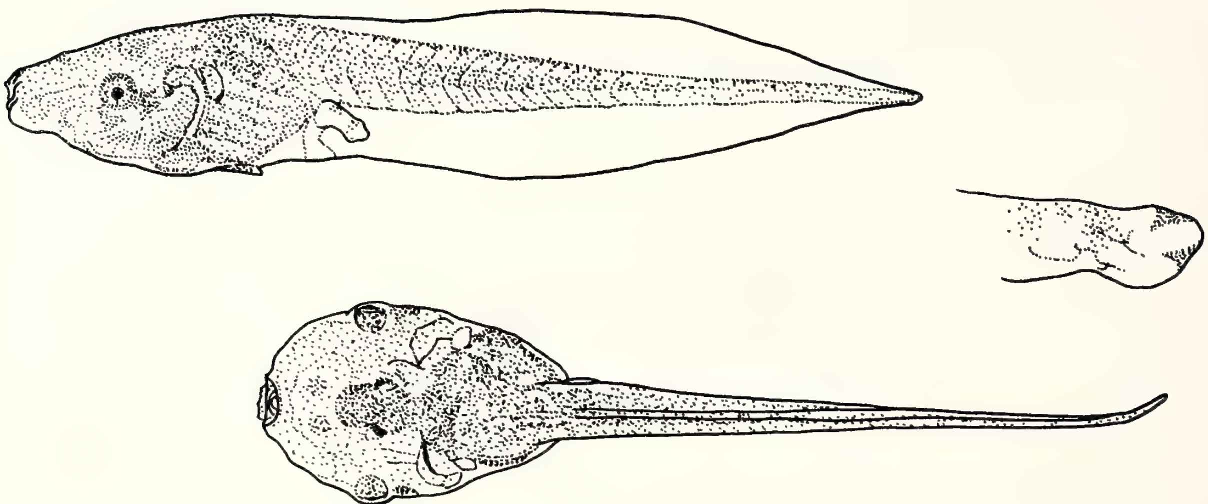

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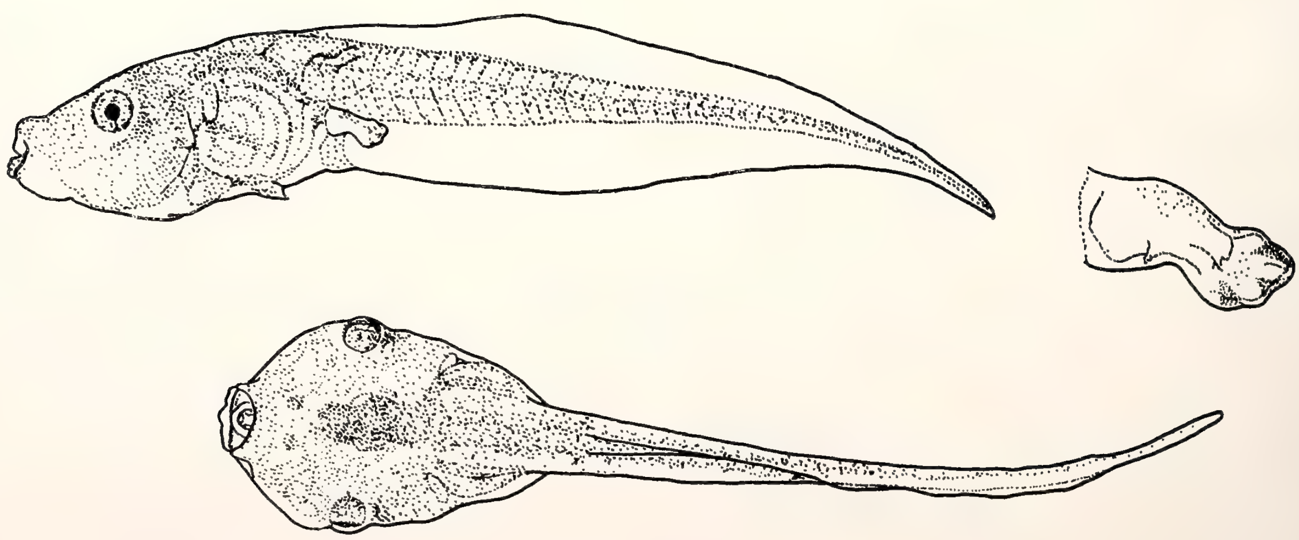

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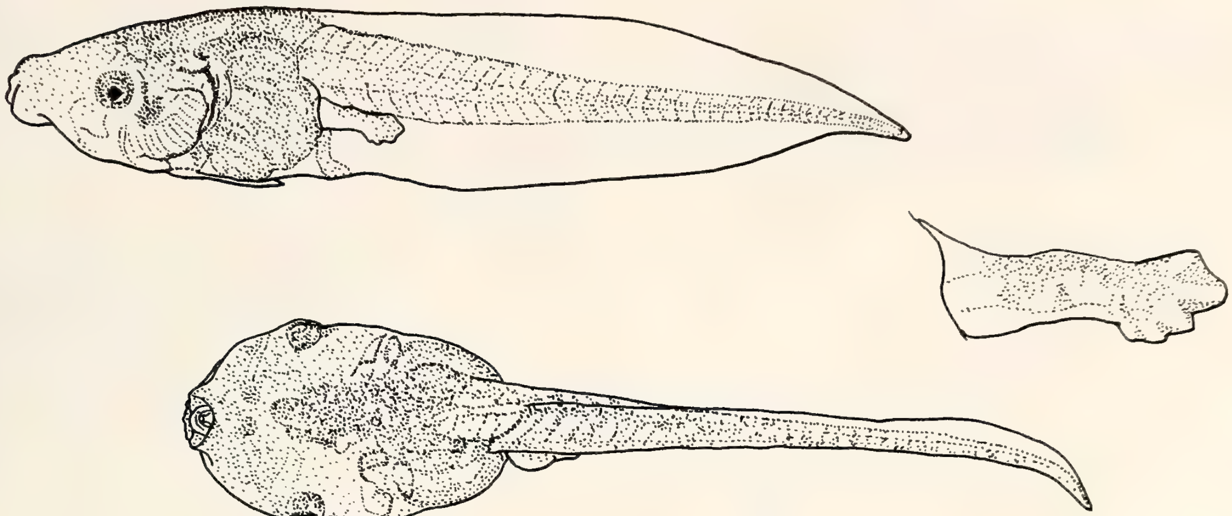
FIG. 1. —Extended.



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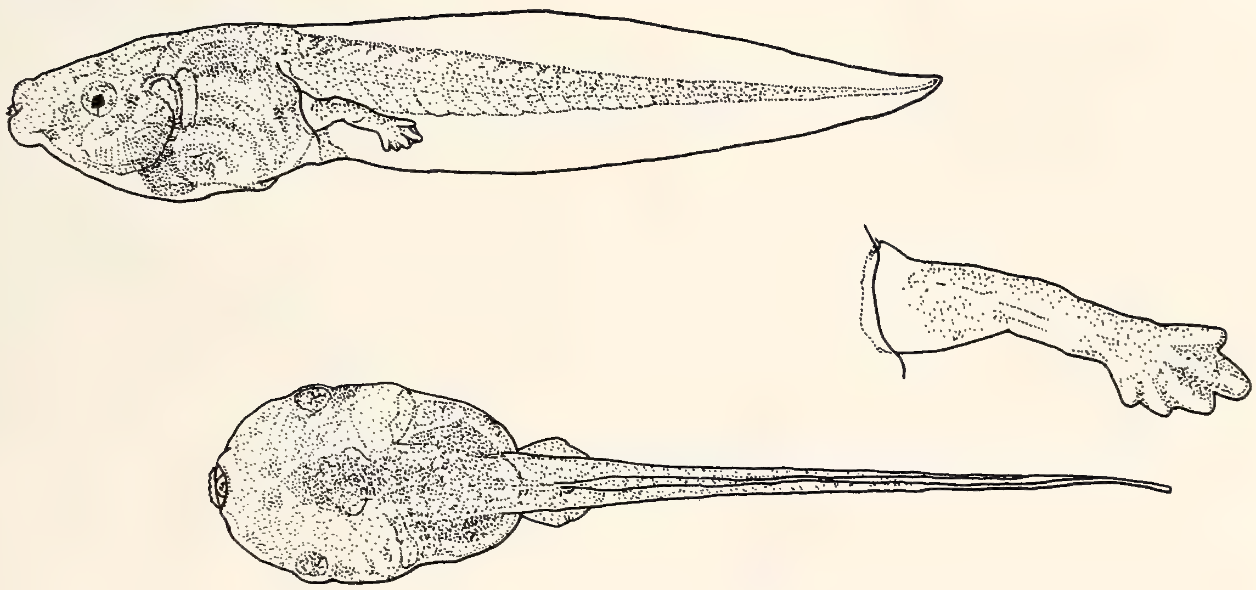

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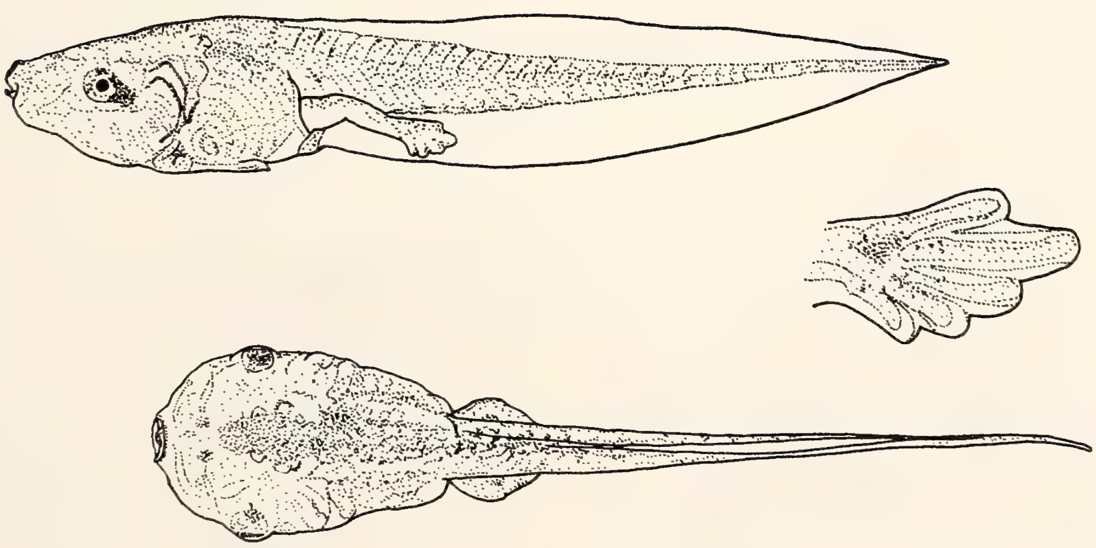

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FIG. 1. —Extended.

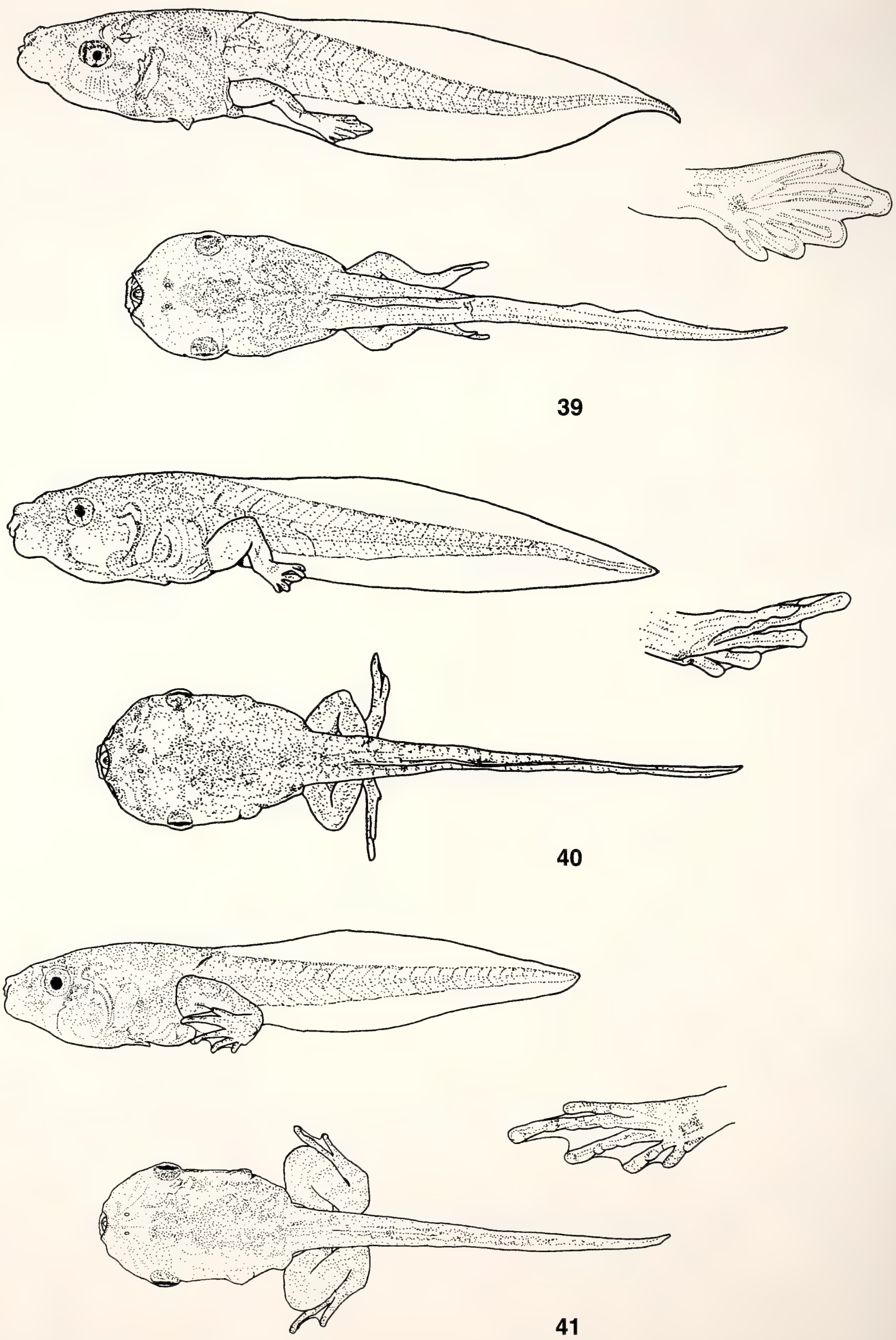

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FIG. 1. —Extended.

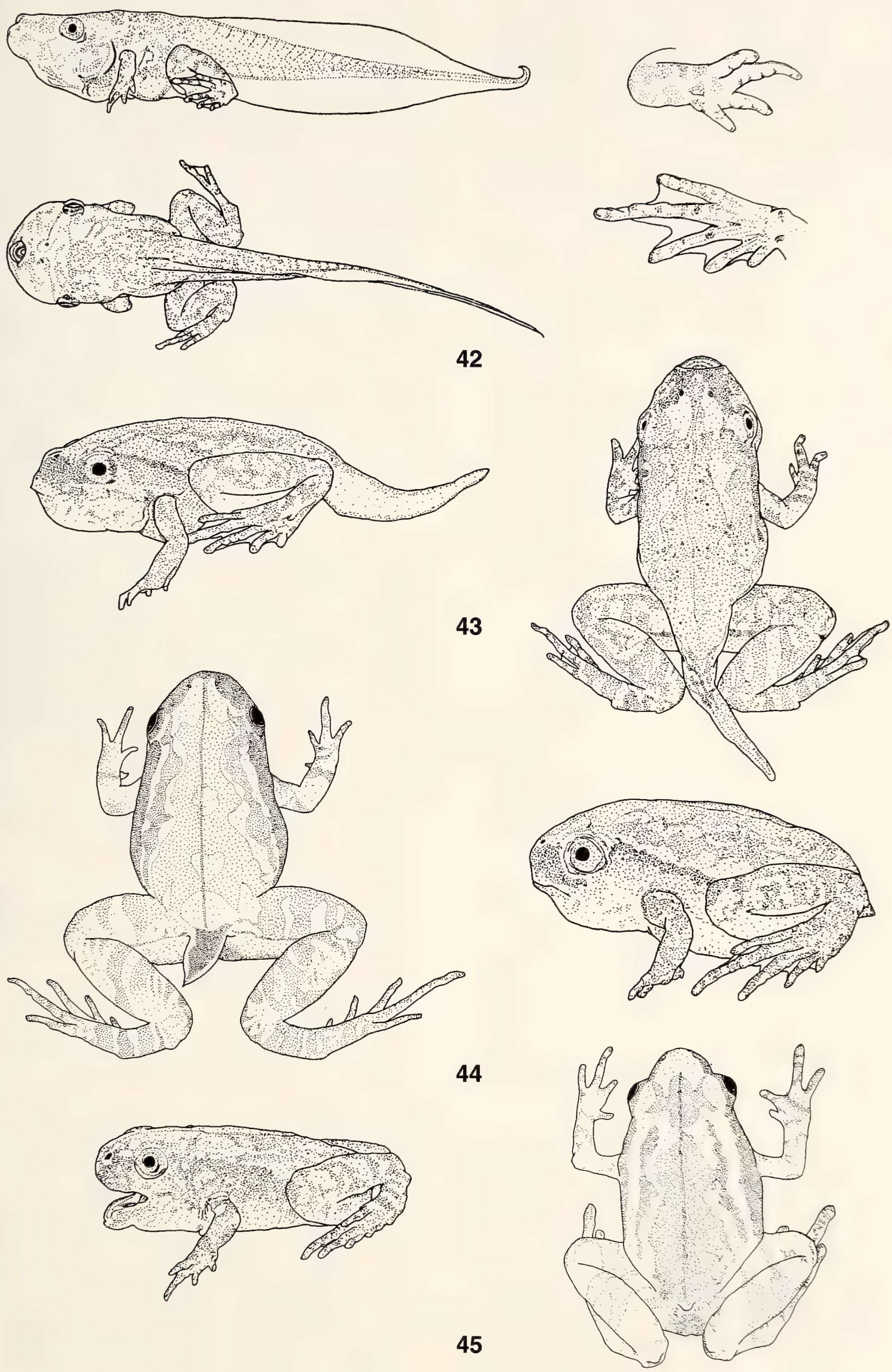

FIG. 1. —Extended.

stages 29–33; Hind limb formation, stages 34–41; and Metamorphosis, stages 42–45.

Throughout the embryonic and larval development, TOL varied considerably (Table 1). The value was quite stable from fertilization (stage 1) to the end of the Gastrula (stage 14). Then it started to increase gradually with the commencement of elongation of the embryo along its longitudinal axis. From stage 22 to stage 27, TOL exhibited a plateau again, but then resumed an appreciable increase, which was particularly remarkable from stages 27 to 29. This is probably due to the commencement of feeding through completion of the functional digestive system. After reaching its peak at stage 40, TOL abruptly dropped to the completion of metamorphosis (stage 45).

Both BL and TAL also peaked at stage 40, and then gradually (BL) or rapidly (TAL) decreased (Appendix I). Mean BL at completion of metamophosis was 7.23 mm (SD=0.48, min=6.32 mm, max=7.94 mm, n=13).

Discussion

The variation in clutch size observed in the present study (220–910) largely overlaps those previously reported for populations from the southern Ryukyus (271–1207: Matsui and Ota, 1984) and Nepal (61–1327; Schleich and Kästle, 2002). However, the egg diameter of the present sample ($\bar{x}$=0.97 mm, SD=0.04, min=0.93 mm, max=1.04 mm) was distinctly smaller than that of the southern Ryukyu populations ($\bar{x}$=1.20 mm, SD=0.03, min=1.0 mm, max=1.3 mm: Matsui and Ota, 1984), and was slightly larger than that of the continental Chinese sample ($\bar{x}$=0.90 mm: Liu et al., 1996).

Compared with Liu et al.'s (1996) description of the embryonic development of the continental Chinese *M. ornata*, the results of our observations also exhibited differences in the timing of a few important changes. Darkening of the stomodium area, for example, was observed at the Neural fold stage (stage 16) in our sample, but was reported to be an event at the Neural tube stage (stage 18) for the continental Chinese sample (Liu et al., 1996).

With respect to tadpole morphology, our sample differs from the Thailand sample in having a silvery sheen in the belly as a result of the appearance of iridescent cells at the Completion of spiracle stage (stage 28) (Khan, 2000). Location of the cloacal tail piece in our sample, medial as in the Taiwanese (Chou and Lin, 1997) and Pakistan (Khan, 2000) samples, differs from that in the Chinese sample, where this organ is reported to be dextral (Liu, 1950).

Further studies are needed to examine the taxonomic implications of these differences (i.e., whether they actually reflect species level differences as predicted by Kuramoto [1976, 1987], Dubois [1987], and Maeda and Matsui [1989], or mere intraspecific variations).

Acknowledgments

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Appendix

Body length (BL) and tail length (TAL: both in mm) (x±SD, followed by ranges in parentheses) of embryos and larvae of Microhyla ornatan *obtained in captivity.*

Stage	n	BL	TAL
28	37	3.51±0.87 (1.62–4.85)	5.05±0.96 (2.79–6.47)
29	5	4.91±0.17 (4.70–5.15)	7.70±0.44 (7.20–8.38)
30	14	5.30±0.40 (4.70–6.03)	7.91±0.35 (7.35–8.53)
31	11	6.00±0.49 (5.15–6.62)	9.70±1.05 (8.23–11.03)
32	18	6.51±0.34 (6.03–7.06)	11.12±0.84 (9.41–12.50)
33	23	6.85±0.32 (6.32–7.35)	11.80±1.15 (9.56–13.67)
34	25	7.47±0.53 (6.62–8.67)	13.61±0.88 (11.91–14.99)
35	8	7.75±0.40 (7.06–8.38)	14.24±0.75 (12.64–15.14)
36	23	8.12±0.43 (7.50–8.97)	16.13±0.86 (14.85–18.67)
37	23	8.44±0.44 (7.50–9.26)	17.02±1.10 (15.29–19.11)
38	12	8.67±0.51 (7.35–9.26)	17.13±0.81 (15.58–18.08)
39	12	8.76±0.58 (7.35–9.56)	17.69±1.23 (15.73–19.40)
40	10	9.08±0.44 (8.38–9.56)	17.73±1.70 (15.14–19.40)
41	15	9.03±0.38 (8.53–10.00)	16.61±1.73 (13.97–19.11)
42	6	8.48±0.48 (7.64–9.11)	13.28±2.65 (8.38–15.29)
43	5	8.35±0.63 (7.79–9.26)	7.24±5.03 (4.00–13.75)
44	5	7.47±0.35 (7.06–7.94)	0.85±1.02 (0.15–2.65)
45	13	7.23±0.48 (6.32–7.94)	0.00±0.00 (—)

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Two Unknown Arboreal Frogs (genus *Platypelis*) Described from the Rainforests of Northeastern Madagascar (Microhylidae: Cophylinae)

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Abstract: Two new arboreal microhylid frogs are described from the rainforests of northeastern Madagascar. *Platypelis tetra* is a very small frog, reaching about 20 mm SVL, and inhabits phytotelms in screw *Pandanus* pines. It was found in some low and mid-altitude rainforests, such as Anjanaharibe-Sud, Besariaka, Tsararano, and Masoala Peninsula. This species diverges from the other *Platypelis* by its small size and colouration, with a series of whitish spots on the back, of which four are more evident. Furthermore, its advertisement call is composed by a long series of discrete notes at about 3.5–4 KHz of frequency, and a repetition rate of about 3.0 notes/s. The second species, *Platypelis mavomavo*, reaches a larger size (about 30 mm SVL), and is characterized by a yellowish colouration of the ventral surface, and a dorsal surface with a network of dark spots on a beige-yellow background. It is currently known from Anjanaharibe-Sud and Ambolokopatrika, around the Andapa water-basin, but is expected to have a wider distribution.

Key words: Amphibia; Anura; Microhylidae; *Platypelis*; New species; Madagascar

INTRODUCTION

Madagascar is well known for its biodiversity and high endemism (Glaw and Vences, 1994). Amphibians are no exception with more than 210 endemic species (Andreone and Luiselli, 2003) and many others still in wait for description. Like other vertebrates of Madagascar (e.g., fish and mammals, see Benstead et al., 2000; Yoder et al., 2003), the frogs of this landmass belong to a few families, of which they represent an offshoot radiation. According to recent studies (Vences and Glaw, 2001) they are included in four families: Ranidae, Mantellidae, Microhylidae, and Hyperoliidae. Of these, the microhylids are still the most enigmatic clade, and their phylogenetic and taxonomic relations are largely unstudied. Of the three recognized Malagasy subfamilies (Dyscophinae, Scaphiophryninae, and Cophylinae), the cophylines account for a high number of species inhabiting rainforests and secondary altitude savannahs (Andreone, 1999). Indeed, little is known of their ecology and distribution

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except that they are specialized to a variety of microhabitats: e.g., the enigmatic *Rhombophryne testudo* and some *Plethodontohyla* species (such as *P. ocellata*, *P. alluaudi*, and *P. serratopalpebrosa*) are fossorial or terrestrial, the small *Stumpffia* are adapted to leaf-litter, and most of the *Anodonthyla*, *Platypelis*, *Cophyla*, and some *Plethodontohyla* are arboreal or semiarboreal. The conditions and evolutionary pressures that led to such a dramatic ecological radiation in these frogs are unknown. Further, whether an ecologically specialised group is indeed monophyletic is the subject of a separate work (Andreone et al., in press).

One reason for the lack of knowledge about cophyline microhylids is that they often lead a cryptic life, which makes their study difficult. In fact, many taxa are known from a few specimens and are only occasionally rediscovered (e.g., *Plethodontohyla coudreaui*; Andreone, in press a). This was confirmed by our own experience: during field surveys we often found specimens that could be grouped with one another representing the same ecological and morphological cluster, but were difficult to assign to any known taxon. This leads us to the idea that, especially in microhylids, many more species await description, and it is not an exaggeration to affirm that most likely the final number of species will stabilize around the double that currently known. Many of these specimens still await description and will be the object of further contributions, using both traditional (e.g., morphological analysis and comparisons of museum samples), and other methods (e.g., acoustics, karyology, DNA). While it is important to collect ecological and natural history information on unknown species, the discovery and rapid description of new frogs from Madagascar is important for two reasons: (1) it accelerates the cataloguing of the whole Malagasy batrachofauna, providing powerful tools to draw biogeographic relationships for conservation actions (see Andreone and Luiselli, 2003), and (2) it helps clarify the taxonomy and phylogeny of this subfamily in a region where deforestation outpaces description. Written records and museum specimens might become all we have for future studies of the most sensitive species, which are rapidly vanishing.

The present paper is just a step in this direction and deals with the description of two new *Platypelis* that we found in northeastern Madagascar. One of these frogs is a *Pandanus*-specialized *Platypelis*, while the second is an arboreal generalist. They are sufficiently divergent from all known *Platypelis*, in both morphology and bioacoustics, to warrant description here.

Materials and Methods

We searched for frogs during the night with the help of flashlights. Vocalizing males were located by following calls at sunset or during rainfall. One species described here is a microhabitat specialist, living within phytotelmata of screw-pines, *Pandanus* sp., and we spent daytime hours searching the leaf axils of these plants. After capture and photography of live colouration, specimens were sacrificed after anaesthesia in chlorobutanol, fixed in 4% formalin or 90% ethanol, and preserved in 70% ethanol. They are currently deposited in the Museo Regionale di Scienze Naturali, Torino (MRSN).

Measurements follow standard methods, using digital callipers or the dissecting microscope's micrometer (to the nearest 0.1 mm): SVL (snout-vent length), HW (head width), HL (head length, from the maxillary commissure to the snout tip), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (inter-narial distance), TD (horizontal tympanum diameter), HAL (hand length, from the carpal-metacarpal articulations to the tip of the longest finger), FORL (forelimb length, from the axilla to the tip of the longest finger), HIL (hindlimb length, from the cloaca to the tip of the longest toe), FOL (foot length, from the tarsal-metatarsal articulations to the tip of the longest toe), FOTL (foot length including tarsus, from the tibiotarsal articulation to the

tip of the longest toe), TIBL (tibia length).

When available, type specimens and/or other representative specimens of presumably related taxa from zoological collections were examined, as well as the original descriptions and subsequent works (e.g., Blommers-Schlösser and Blanc, 1991; Glaw and Vences, 1994). The advertisement calls were recorded with a SONY TC-D3 tape recorder with external microphone and analyzed with a VOXYS 3.1 sound system (Andreone et al., 2003). The proposed common names for the new species are provided in the authors' original languages (Italian and English) and in Malagasy.

Platypelis tetra sp. nov.

Four-spotted tree cophyline frog (English), cofilìno degli alberi dalle quattro macchie (Italian), sahonkely anatihazo misy tebok'efatra (Malagasy).

Figs. 1–3

Type series

Holotype: MRSN A2174, adult male, Campsite W2, Anjanaharibe-Sud Massif, Analabe Valley, Befandriana Fivondronana, Mahajanga Faritany (Majunga Province), 14°46'S, 49°26'E, 1,250 m asl, collected by F. Andreone, H. Randriamahazo, and J. E. Randrianirina, 5 February 1996. Paratypes: MRSN A2171–2173, three adult females, same data as the holotype; MRSN A1952–1953, an adult male and an adult female, Tsararano Forest, Andatony Anivo, Andapa Fivondronana, Antsiranana Faritany (Diégo Suarez Province), 14°54.8'S, 49°42.6'E, 600 m asl, collected by F. Andreone and J. E. Randrianirina, 5 and 10 December 1996; MRSN A2175, one juvenile, Besariaka Forest, Andapa Fivondronana, Antsiranana Faritany (Diégo Suarez Province), between 14°49'S–14°50'S, 49°35'E–49°36'E, about 700 m asl, collected by J. E. Randrianirina, 1 May 1996; MRSN A646.1–4, three adult males and one juvenile, Masomihenjina Forest, Masoala Peninsula, Maroantsetra Fivondronana, Toamasina Faritany (Tamatave Province), 15°25'S, 49°46'E, collected by R. Nincheri, 24 July 1993; MRSN A2161, A2164–2165, one juvenile and two adult males, Ambohidroina Forest, Masoala Pensinsula, Mahalevona Fivondronana, Toamasina Faritany (Tamatave Province), 15°26.00'S, 49°57.56'E, 840 m asl, collected by J. E. Randrianirina, 26 January 2002; MRSN A2166, one juvenile, same date and collector, 28 January 2002; MRSN A2167, an adult male, same date and collector, 29 January 2002; MRSN A2169, one juvenile, same date and collector, 30 January 2002; MRSN A2170,

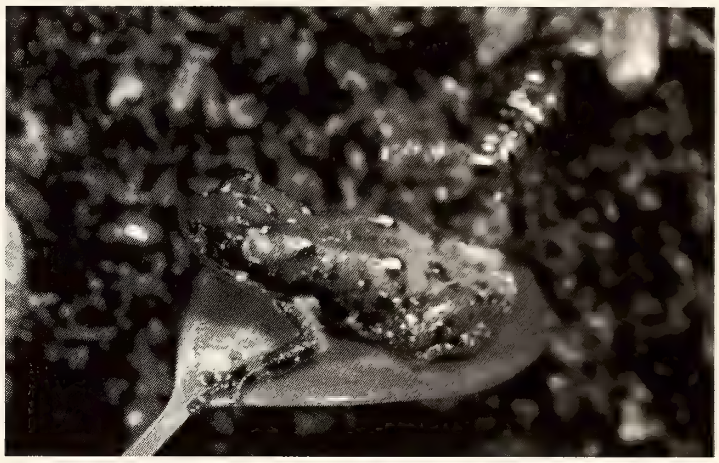

FIG. 1. *Platypelis tetra*. Holotype (male, MRSN A2174) from Analabe Valley, Anjanaharibe-Sud, western slope, NE Madagascar.

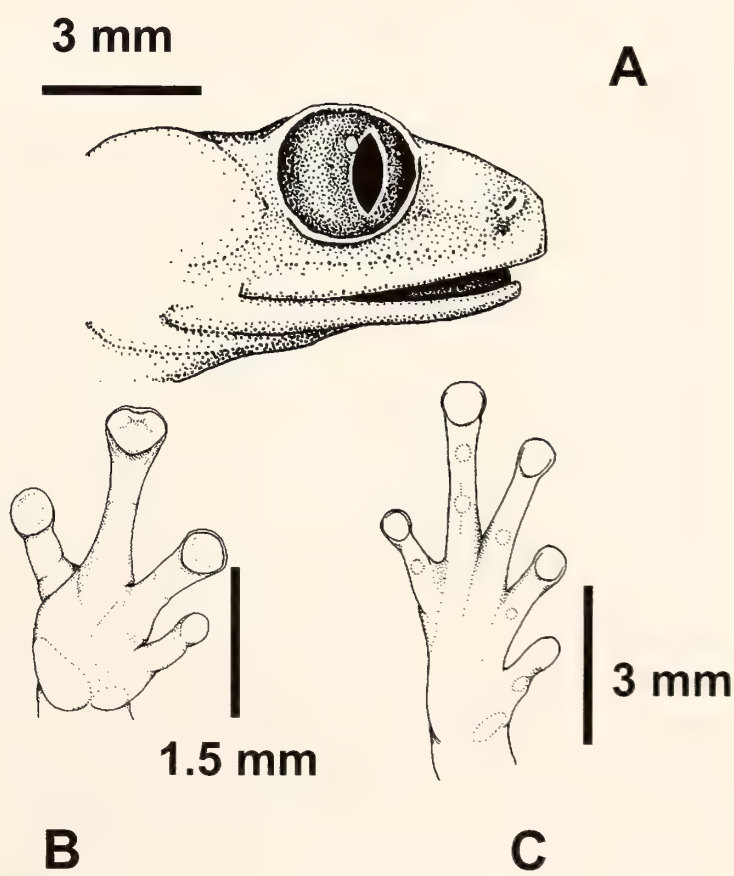

FIG. 2. Particular of the head (A), hand (B), and foot (C) of the holotype of *Platypelis tetra* (male, MRSN A2174).

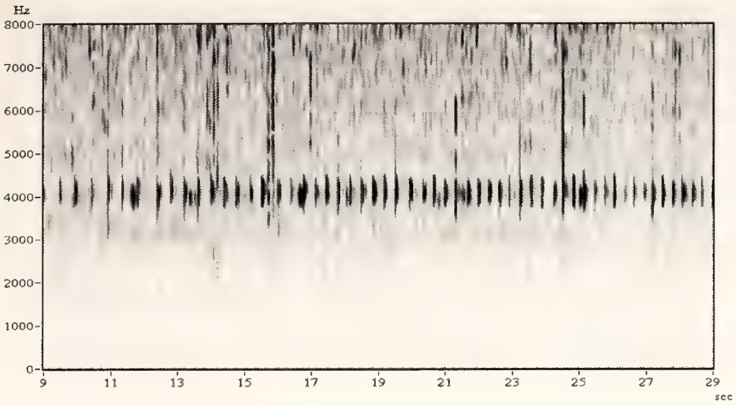

FIG. 3. Sonagram of part of a call (two notes out of a longer note series) of *Platypelis tetra*, recorded at Anjanaharibe-Sud, NE Madagascar. Recording temperature: 18C.

same date and collector, 9 February 2002.

Diagnosis

An arboreal *Platypelis* characterized by the following combination of characters: small adult body size, canthus rostralis indistinct, toe and finger pads more circular than ovoid and moderate in size; hands and feet without webbing, tarso-metatarsal articulation reaching the eye, dorsal surface smooth, dorsal colour shading from brown to tan, sometimes with irregular markings and at least four oblong white spots on the dorsal surface.

Description

The SVL in the examined males ranged from 15.7 to 18.2 mm. In the females from 17.5 to 19.4 mm. Other measurements of the type series are given in Table I. Head as long as wide; HW 32–41% of SVL; HL 31–41% SVL; snout short, slightly protruding beyond margin of lip; rounded and blunt in dorsal view and in profile; END less than ED; END 20–26% of HL; eye moderate in size; ED 28–36% of HL. Top of head flat; cranial crests absent; canthus rostralis indistinct; internarial area not depressed; nostril more circular than ovoid; protruding laterally. Weak supratympanic fold usually visible, tympanum round, TD 34–67% of ED. Choanae small, round, separated, partially obscured by palatal shelf of maxillary arch. Vomerine teeth present and posterior to choanae. Tongue trapezoid in shape with rounded edges, widest at the free margin, no groove or notch, free behind for about two thirds of its length. Pupil horizontal. Vocal sac moderate in size, single, and in gular position. Skin on dorsum of head, body, belly, and limbs smooth. Fingers moderate in length bearing circular, moderately sized discs compared with phalanges; disc on first digit smaller than others: 1<2<4<3; subarticular tubercles faint, circular, not elevated; supernumerary tubercles absent; palmar tubercle semi-distinct, not elevated. No webbing between fingers. Feet with semi-distinct supernumerary tubercles; vestiges of metatarsal tubercle; no other distinct tubercles. Small pads on toes, circular in shape. No webbing between toes. Toe length: 1<2<5<3<4.

Coloration

After five-seven years of preservation in ethanol, all the specimens still show contrasting colouration. Dorsum of head, body, and limbs are brown or tan with vague darker markings, and legs have irregular tan spots. Four bigger dorsal whitish spots are present on the back (two just behind the head, and two other on the sacral bone). Other smaller scattered light points are also present of the back and superior parts of legs in some individuals. In some cases the four main spots form a sort of hourglass figure on the dorsum. Flanks with a brown dorsolateral band beginning posterior to the eye, passing through the union of the arms with the body and down each flank, ending at the groin; brown bar is edged above and below by a thin cream line. The anterior surface of the arm with a brown line extending from the union of the arms with the body and terminating at or before the elbow. Iris brownish shading to copper, with peripheral fine spots and dark shadings. Ventral surface grey to creamish with a few brown flecks, sometimes forming dark spots.

Variation

The paratypes come from Anjanaharibe, Besariaka, Tsararano, and Masoala, spanning therefore about 200 km. These specimens agree with the holotype in colouration. The

TABLE 1. Morphometric measurements (in mm) of type specimens of *Platypelis tetra* sp. nov. and *P. mavomavo* sp. nov. M=male, F=female, J=juvenile, holotype marked with an asterisk. For abbreviations see the text.

Museum number	Provenience	Sex	SVL	HW	HL	ED	END	NSD	NND	TD	HAL	FORL	FOL	TIBL
Platypelis tetra														
MRSN A2174*	Anjanaharibe-Sud	M	17.5	6.4	6.2	2.0	1.3	1.6	2.0	0.8	3.2	5.9	6.0	17.5
MRSN A2172	Anjanaharibe-Sud	M	18.2	6.5	6.3	2.2	1.4	1.6	2.1	1.2	3.4	6.5	5.4	18.2
MRSN A1952	Tsararano	M	16.5	5.9	5.8	1.9	1.3	1.5	2.0	0.9	2.8	6.6	4.5	16.5
MRSN A646.1	Masomihenjina	M	15.8	5.5	5.7	1.9	1.4	1.4	2.0	0.9	3.1	6.4	5.3	15.8
MRSN A646.2	Masomihenjina	M	15.7	6.2	6.1	2.1	1.2	1.4	2.0	1.0	3.4	6.4	5.2	15.7
MRSN A646.3	Masomihenjina	M	16.2	5.2	5.8	1.8	1.5	1.5	2.1	1.1	2.8	5.9	4.6	16.2
MRSN A2164	Ambohidroina	M	18.2	6.2	6.3	2.2	1.5	1.7	2.0	0.9	3.4	6.6	5.4	18.2
MRSN A2165	Ambohidroina	M	18.2	6.8	6.3	1.8	1.4	1.9	2.2	1.2	3.7	7.3	5.7	18.2
MRSN A2167	Ambohidroina	M	15.9	5.7	5.5	1.7	1.4	1.5	2.2	1.1	2.9	6.5	4.5	15.9
MRSN A2170	Ambohidroina	M	16.6	5.9	5.7	2.0	1.4	1.7	2.1	1.0	2.8	6.7	5.5	16.6
MRSN A2173	Anjanaharibe-Sud	F	19.4	6.8	7.1	2.3	1.5	1.6	2.1	0.8	3.8	6.3	5.9	19.4
MRSN A2171	Anjanaharibe-Sud	F	18.9	6.9	6.4	2.3	1.5	1.9	2.2	1.2	4.1	7.6	5.7	18.9
MRSN A1953	Tsararano	F	17.5	5.8	6.3	2.0	1.3	1.8	2.1	1.0	2.8	6.8	5.1	17.5
MRSN A2175	Besariaka	J	13.1	5.3	5.3	1.9	1.1	1.3	1.8	1.0	2.9	6.5	4.9	13.1
MRSN A646.4	Masomihenjina	J	11.5	4.5	4.7	1.5	1.1	1.3	1.7	0.7	2.1	4.1	3.9	11.5
MRSN A2161	Ambohidroina	J	14.3	5.2	5.4	1.9	1.3	1.3	1.7	0.7	3.1	6.5	4.3	14.7
MRSN A2166	Ambohidroina	J	14.9	5.3	5.5	2.0	1.4	1.3	1.8	0.7	3.2	6.4	4.3	14.9
MRSN A2169	Ambohidroina	J	14.9	5.2	6.1	1.8	1.2	1.5	1.8	0.8	3.2	6.0	4.2	14.9
Platypelis mavomavo														
MRSN A2435*	Ambolokopatrika	M	26.5	9.3	9.7	4.1	2.5	2.1	3.0	1.7	8.1	18.8	12.4	26.5
MRSN A2432	Ambolokopatrika	M	28.6	11.3	9.6	3.6	2.5	2.4	2.7	1.7	8.6	20.7	12.6	28.6
MRSN A2434	Ambolokopatrika	M	24.4	8.4	8.5	2.9	2.5	2.1	2.6	2.1	8.8	17.5	10.7	24.4
MRSN A2431	Ambolokopatrika	F	27.9	9.9	9.7	4.0	2.4	2.6	3.2	1.8	8.5	18.3	12.8	27.9
MRSN A2433	Ambolokopatrika	F	26.8	9.4	8.8	3.4	2.0	2.4	3.6	1.6	8.1	19.4	11.7	26.8

individuals from Masomihenjina (Masoala) show a paler dorsal colouration, and a contrasted lateral band. Colouration in life was basically similar to that described for preserved animals, although much more vivid.

Etymology

The word "tetra" is derived from the Greek "tetras" means "four" and is attributed to the fact that most specimens show four lighter spots on the back. This name is used as a noun with the same meaning.

Natural history and distribution

The holotype and some of the paratypes were collected at Anjanaharibe-Sud, western slope (Analabe Forest), Campsite W2 (around 1200 m asl), where the habitat is comprised of a mid-high altitude transitional rainforest with tall trees and abundant lichens, mosses, and ferns (Raxworthy et al., 1998; Andreone et al., 2000). The specimens were found within a plant of the genus *Pandanus*. A single specimen was found at Besariaka Forest. This forest at about 60 km south of Andapa is rather degraded, especially in areas far from streams, due to cattle, cutting of trees by

villagers, and use of path systems. The specimen was found within a *Pandanus*, and did not call. Other paratypes came from Tsararano. The mountain chain and forest of the same name lie midway between the Anjanaharibe-Sud Massif and the Masoala Peninsula, and is formed by several hills. The forest was at that time (1996) mostly intact, perhaps due to its distance from large-sized villages. The remainder of specimens were found at Masoala at two low altitude sites (Masomihenjina and Ambohidroina), always within *Pandanus*. This species is evidently specialized to this plant, and is a phytotelm frequenter (Lehtinen, 2003). Concerning the distribution, *P. tetra* was until now found only in the northeastern sector of Madagascar, an area which is interesting in terms of endemicity and species diversity (Andreone, in press b). We do not have evidence of a wider distribution, although we strongly suspect that its presence runs southward, and possibly, northwards.

Advertisement calls

Calls were recorded on 5 February 1996 between 1830 and 1900 h, at an air temperature of 18.0–18.5C. They consisted of an almost endless series of chirping metallic notes. Temporal call parameters were: note duration 171–318 ms ($\bar{x}\pm SD=266\pm41$ ms, N=17), duration of inter-note intervals 148–398 ms (226 ± 59 ms, N=13), note repetition rate 3.0/s. The frequency showed a simple pattern, with a dominant frequency between 3500–4000 Hz.

Comparisons with other species

Of the other *Platypelis* species possibly sympatric and similarly sized, *P. tetra* differs: (1) from *P. barbouri* in lacking webbing between the toes and fingers, in lacking the red or green markings on the dorsal surface of the groin and thighs, in the presence of white dorsal spots, and of a brown dorsolateral bar bordered in cream; (2) from *P. cowani* in lacking webbing between the toes, in the absence of a beige triangular marking on the dorsal surface, in the possession of white dorsal spots, and brown dorsolateral bars; (3) from *P. occultans* in lacking webbing between the toes, a triangular marking on the back, in a tibio-tarsal articulation extending to the eye, in the possession of dorsal spots, and in the presence of the dorsolateral bands. Within the wealth of *Platypelis* species, *P. tetra* is morphologically similar to *P. tuberifera*, although the latter reaches a larger size (up to 40 mm). *Platypelis tetra* could be misidentified with juveniles of this species, from which it differs in lacking toe webbing, the absence of a dorsal median line, the possession of white dorsal spots, and in the possession of a brown dorsolateral bar bordered in cream.

Platypelis mavomavo sp. nov.

Yellowish tree cophyline frog (English), cofilìno arboreo giallastro (Italian), sahonkely mavomavo (Malagasy)

Figs. 4–6

Type series

Holotype. MRSN A2435, adult male, Ambolokopatrika, Antsinjorano, 14°32.6'S, 49°25.8'E, 975 m asl, Andapa Fivondronana, Antsiranana Faritany (Diégo Suarez Province), collected by F. Andreone, G. Aprea, and J. E. Randrianirina, 10 December 1997; Paratypes. MRSN A2433, an adult female, same data and collectors, 18 December 1997; MRSN A2431, adult female, Ambolokopatrika, Andemakatsara, 14°31.8'S, 49°26.5'E, 875 m asl, collected by F. Andreone and J. E. Randrianirina, 2 June 1997; MRSN A2432, adult male, Ambolokopatrika, Andranomadio, 14°32.4'S, 49°26.3'E, 890 m asl, collected by F. Andreone, G. Aprea, and J. E. Randrianirina, 1 December 1997; MRSN A2434, adult male (dissected for karyological analyses), Ambolokopatrika, Antsinjorano, collected by F. Andreone, G. Aprea, and J. E. Randrianirina, 1 December 1997.

Other specimens

Platypelis mavomavo was first discovered in January 1996 at Anjanaharibe-Sud, western

FIG. 4. *Platypelis mavomavo*. Holotype (male, MRSN A2435), dorsal view, from Ambolokopatrika, NE Madagascar.

FIG. 5. Particular of the head (A), hand (B), and foot (C) of the holotype of *Platypelis mavomavo* (male, MRSN A2435).

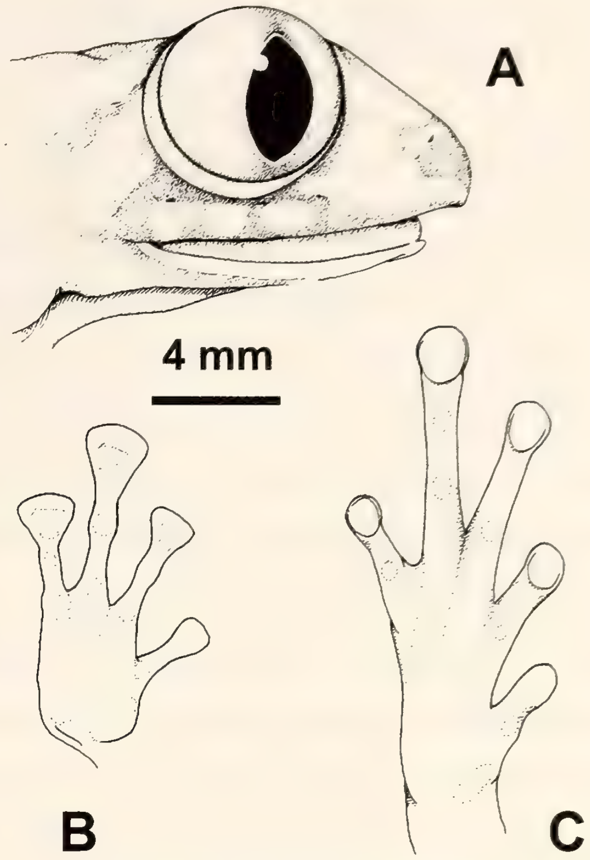

FIG. 6. *Platypelis mavomavo*. Holotype (MRSN A2435), ventral view.

slope. The specimen discovered was photographed but not captured.

Diagnosis

An arboreal species of *Platypelis* characterized by the following combination of characters: medium adult body size (SVL=35 mm), yellowish colouration, with extended brownish marblings, digital expansions triangular in the fingers and ovoid on toes, vestigial webbing between the toes, dorsal surface from smooth to slightly warty.

Morphological description

The three males ranged in size from 24.4 to 28.6 mm; the two females were respectively 26.8 and 27.9 mm long. Other measurements of the type series are given in Table I. Head as long as wide; HW 34–40% of SVL; HL 33–35% SVL; snout short, slightly protruding beyond margin of lip; rounded in dorsal view (in profile, lightly truncated); END less than ED; END 23–30% of HL; eye moderate in size; ED 34–42% of HL. Top of head flat; cranial crests absent; canthus rostralis indistinct; internarial area not depressed; nostrils protruding dorsally and laterally. Supratympanic fold visible, tympanum round, TD 41–47% of ED. Choanae small, round, and separated. Vomerine teeth present and posterior to choanae. Tongue trapezoid in shape with rounded edges, widest at the free margin, with a slight frontal notch. Vocal sac moderate in size, single, and in gular position. Skin on dorsum of head, body, and limbs quite smooth, although in some specimens elongated warts are visible. Ventral surfaces smooth. Forearm broad, fingers moderate in length bearing subtriangular, large discs; disc on first digit smaller than others: 1<2=4<3; subarticular tubercles faint, circular; supernumerary tubercles absent; palmar tubercle semi-distinct, not elevated. No webbing between digits of hands. Feet with semi-distinct supernumerary tubercles. Small pads on digits of feet, ovoid in shape. Vestigial webbing between third and

fourth, and between fourth and fifth toes. Toe length: 1<2≤5<3<4.

Coloration

After about six years in preservative solution, the dorsum of this new *Platypelis* has become light brownish, with scattered lighter spots and reticulations. The legs have approximately the same colouration as the back, with some pigmented transverse areas or spots. Most of the hands and feet are brown or black. Pupil horizontal, iris yellowish with dark spots. The ventral surface is cream/yellow with some faint dark marbling, more visible on the throat. In life, the colouration is similar in pattern, although more distinct. The yellow is conspicuous and bright in live animals (especially on the belly), but disappeared after preservation in formalin and/or ethanol.

Variation

The type series consists of four specimens from Ambolokopatrika, quite similar in morphology and size to the holotype.

Advertisement calls

Unknown.

Etymology

The specific name *mavomavo* (pronounce: "mow-woo mow-woo") is a Malagasy term: "mavo" means yellow, and the duplicate "mavomavo" adjective yellowish. It makes obvious reference to the typical species' colouration and is used as an invariable noun standing in apposition to the generic name.

Natural history and distribution

The type series was collected at Ambolokopatrika, where the habitat is a mid-high altitude transitional rainforest, with tall trees and an abundance of epiphytic plants. The specimens were found during rainy nights climbing well above the ground (about 2–4 m) on small tree trunks. A similar habitat was observed for one specimen (not captured) at Anjanaharibe-Sud. This species is apparently not a phytotelm specialist, although it is likely that, similarly to other species (e.g., *P. pollicaris*) it may hide during the day and dry periods in trunk-holes, under barks, and at the basis of epiphytic plants. Given its presence at Ambolokopatrika and Anjanaharibe-Sud, we expect the species' distribution to include other areas of northeastern Madagascar.

Comparisons with other species

Platypelis mavomavo is most similar to *Platypelis cowani* and *P. pollicaris*. They are different from *P. mavomavo* especially in colouration. In particular, both have a brown dorsum, with light spots, and their belly is typically white. *Platypelis tsaratananaensis* (at least the specimens from Anjanaharibe-Sud) has a more slender body, and it hides in bamboo internodes.

Discussion

The discovery of the new *Platypelis* species described herein, at some localities of northeastern Madagascar highlights the incomplete knowledge of the microhylid fauna of Madagascar. *Platypelis tetra* is likely the smallest arboreal frog of Madagascar, reaching a SVL of only 18 mm. It is smaller than *P. barbouri* (23 mm), *Anodonthyla boulengeri* (23 mm), and *A. nigrigularis* (24 mm) (records from Glaw and Vences, 1994). This small size coupled with sticky skin and toe pad shape makes *Platypelis tetra* a good climber of the leaves beneath which it lives. At Anjanaharibe-Sud we found *Platypelis tetra* in syntopy with another *Platypelis* of small size (*P. occultans*), *P. tuberifera*, *Mantidactylus* cf. *bicalcaratus* and *M. pulcher*.

Platypelis tetra lives in close relationship with the *Pandanus* microhabitat, and, therefore, needs a pristine or mature secondary rainforest. *Platypelis mavomavo* was found only at some intact forest fragments of Anjanaharibe-Sud and Ambolokopatrika forests. In this sense, both these cophylines are both closely tied to the existence of an intact rainforest and to high humidity levels. For these reasons they are good candidates for being

ecologically sensitive species, as stressed by Vallan (2000) and Andreone and Luiselli (2003).

Given the increasing deforestation occurring in the Andapa water basin (Berner, 1995), the protection of the remnant forest fragments corresponds to the protection of the *Pandanus* screw pines where *P. tetra* and other frogs live. The sites where the new species were found are either partly included in a reserve network or are in places where logging is still conducted at a low rate. As outlined by Vallan et al. (in press) this kind of forest exploitation does not have an immediate negative effect on the batrachofauna. The long-term effects are still largely unknown, but it is obvious that the physical reduction of the rainforest will coincide with the disappearance of many frog species.

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Titles of Papers Presented at the 42nd Annual Meeting of the Herpetological Society of Japan (25–26 October 2003 at Toho University, Funabashi)

Abstracts in Japanese will appear in Bulletin of the Herpetological Society of Japan, Vol. 2004, No. 1.

SYMPOSIUM: Advances in the Biological Studies on Snakes—Toward an Integration of Ethological, Ecological, and Molecular Approaches to Their Evolution

1. Molecular evolution of crotaline snake venom components. Motonori Ohno
2. Another "poisonous" gland in snakes: function, ultrastructure, and physiology of the nuchal glands of *Rhabdophis tigrinus*. Akira Mori

GENERAL SESSION

[Turtles]

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2. Radiotelemetric study on movements of *Mauremys japonica* and *Chinemys reevesii* for egg laying and hibernation in the Kizu Rriver. Machiko Okuyama and Akira Mori
3. Habitat use and activity of the common snapping turtle, *Chelydra serpentina*, studied by thermal data logging. Raita Kobayashi and Masami Hasegawa
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5. Preliminary report of endoparasites obtained from two alien turtle species (*Trachemys scripta* and *Chelydra serpentita*) in Japan. Aya Ishida, Hajime Iwao, Mitsuhiko Asakawa, Masami Hinoue, Shintaro Abe, and Raita Kobayashi
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8. Taxomic status of *Tropidophorus cocincinensis* Duméril et Bibron, 1839 and *T. microlepis* Günther, 1861 in Indochina. Tsutomu Hikida
9. Preliminary study on comparative osteology of Malagasy scincine skulls. Shuichi Sakata and Tsutomu Hikida
10. Annual rings in bones of *Anolis carolinensis* from the Ogasawara Islands. Isamu Okochi, Ayumi Okada, Takashi Ohbayashi, Shingo Miura, and Hiroki Sato
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12. Geographic variation in *Takydromus tachydromoides* from Kyushu and adjacent regions. Naofumi Takahashi and Hidetoshi Ota
13. Origin of assemblages of the parthenogenetic gecko, *Lepidodactylus lugbris*, in Japan: a molecular perspective. Saiko Yamashiro, Masanao Honda, and Hidetoshi Ota
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15. Seasonal changes in nocturnal activity of the Tawa gecko, *Gekko tawaensis* from a Shinto Shrine, Hiroshima Prefecture, Japan. Tatsuya Hara and Sumio Okada

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17. Study on the ecosystem sustaining a pair of golden eagles: Variations of snake species composition and diet comparison among habitats. Akira Mori, Hajime Moriguchi, and Masami Hasegawa
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19. Study on the ecosystem sustaining a pair of golden eagles: Snake operative temperature and its use for estimating duration of above-ground snake activities. Masami Hasegawa, Akira Mori, Hajime Moriguchi, and Mariko Nishimura
20. Thoughts on the origin and function of crotaline pit organs. Richard C. Goris, Yoshitoshi Atobe, and Kengo Funakoshi
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[Reptiles in general]

29. Hybrids between native and exotic reptiles of the Ryukyus, Japan. Hidetoshi Ota, Hiroyuki Sato, Mamoru Toda, Yuichirou Yasukawa, and Dai Hatanaka

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80. A review of the recent status of amphibian and reptile population in southern Russian Far East. Maslova Irina

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The cover page should show the title, and name and postal address of the corresponding author to whom communications regarding the manuscript should be directed with his/her telephone and fax numbers, and e-mail address. The cover page should also show the date of submission, the numbers of pages of main text and figure legends, and the numbers of figures and tables.

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HIRATE, K. 2000. Species Composition, Sex Ratio, Size Distribution, and Seasonal Fluctuation of the Sea Turtle Assemblage in the Eastern Off-shore of the Central Part of Okinawajima. Unpublished master's thesis. University of the Ryukyus, Nishihara. (in Japanese)

HONDA, M, Y. YASUKAWA, AND H. OTA. In press. Phylogeny of the Eurasian freshwater turtles of the genus Mauremys Gray, 1869 (Testudines). J. Zool. Syst. Evol. Res.

KAMEZAKI, N. 1989. The nesting sites of sea turtles in the Ryukyu Archipelago and Taiwan. p. 342-348. In: M. Matsui, T. Hikida, and R. C. Goris (eds.), Current Herpetology in East Asia. Herpetological Society of Japan, Kyoto.

LEVITON, A. E. AND R. H. GIBBS, Jr. 1988. Standards in herpetology and ichthyology. Standard symbolic codes for institution resource collections in herpetology and ichthyology. Supplement no. 1: additions and corrections. Copeia 1988: 280-282.

LEVITON, A. E., R. H. GIBBS, Jr., E. HEAL, AND C. E. DAWSON. 1985. Standards in herpetology and ichthyology: part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985: 802-832.

MATSUI, M. 1987. Isozyme variation in salamanders of the nebulosus-lichenatus complex of the genus Hynobius from eastern Honshu, Japan, with a description of a new species. Jpn. J. Herpetol. 12: 50-64.

MATSUI, M., H. IWASAWA, H. TAKAHASHI, T. HAYASHI, AND M. KUMAKURA. 1992a. Invalid specific status of Hynobius sadoensis Sato: electrophoretic evidence (Amphibia: Caudata). J. Herpetol. 26: 308-315.

MATSUI, M. AND K. MIYAZAKI. 1984. Hynobius takedai (Amphibia, Urodela), a new species of salamander from Japan. Zool. Sci. 1: 665-671.

MATSUI, M., T. SATO, S. TANABE, AND T. HAYASHI. 1992b. Electrophoretic analyses of systematic relationships and status of two hynobiid salamanders from Hokkaido (Amphibia: Caudata). Herpetologica 48: 408-416.

MOODY, S. M. 1980. Phylogenetic and Historical Biogeographical Relationships of the Genera in the Family Agamidae (Reptilia: Lacertilia). Unpublished doctoral dissertation. University of Michigan, Ann Arbor.

TAKENAKA, T. 2000. Extinction of the naturalized freshwater turtle in Chichijima-Island

of Ogasawara (Bonin) Islands, South Japan. Bull. Herpetol. Soc. Japan 2000: 4-7. (in Japanese with English abstract)
ZHAO, E. AND K. ADLER. 1993. Herpetology of China. Contribution to Herpetology, 10. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

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Detailed information not essential to the text but important to subsequent evaluation (such as that regarding specimens examined) may be placed under the major heading, APPENDIX, and an appropriate sub-subheading. When institutional abbreviations are used in APPENDIX, and/or in the main text, it is recommended, as far as possible, to follow Leviton et al. (1985) or Leviton and Gibbs (1988) (see example of references above for detailed information on these publications) with an explicit statement in APPENDIX (or otherwise, in MATERIALS AND METHODS of the main text): e.g.,

APPENDIX

Specimens examined

Catalogue numbers of specimens deposited in the zoological collection of Kyoto University Museum are preceded by KUZ. The other acronyms are those suggested by Leviton et al. (1985).

Geoemyda japonica: Okinawajima, Okinawa Pref., Japan, KUZ R36720, NSMT H02083-02086; Kumejima, Okinawa Pref., Japan, KUZ R36721, OMNH-R3334. G. spengleri: Vietnam, NSMT H9999, ...

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Kyoto University, Sakyo-ku, Kyoto, Japan, 23–24 October 2004
(Masafumi Matsui, Chair)

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Structure of Shell Membranes and Water Permeability in Eggs of the Chinese Soft-shelled Turtle *Pelodiscus sinensis* (Reptilia: Trionychidae)

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Abstract: The shell membrane of a Chinese soft-shelled turtle egg acts as a bag enclosing egg white and water. The main body of the shell membrane is a meshwork of fibers, and is organized into an inner and an outer membrane. The limiting membrane demarcates the shell membrane at the interface with the egg white. Transmission electron microscopy revealed that the limiting membrane has a width of 362 nm and consists of dense materials. There were many canals that run perpendicularly or obliquely to the plane of the membrane. Scanning electron microscopy showed many holes, 20 nm in size, on the inner surface of the membrane. The water permeability of the shell membrane was 5 nl/mm^2 per min. Neither the ultrastructure nor water permeability changed throughout the incubation period of 54 days. No significant difference was observed between membranes of the embryonic side and yolk side of individual eggs. These results are compared with those of corresponding observations of avian eggs.

Key words: Chinese soft-shelled turtle; Shell membrane; Limiting membrane; Ultrastructure; Water permeability

INTORODUCTION

For eggs that are laid on land, it is important that a certain amount of water be maintained around the embryos to protect them from drying out. In addition to the outermost eggshell, avian eggs possess a limiting membrane, a component of the shell membrane which retains the water in the yolk and egg white in the early stages of development. However, at the late stages of development, avian embryos need to take up calcium from the eggshell for bone formation (Johnstone and Comar, 1955; Simkiss, 1961; Ono and Wakasugi, 1984) and need to dry out for initiation of breathing. To achieve this, avian embryos produce a thin limiting membrane with an as yet unknown agent, thus enabling acceleration of water

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permeation, and the parent birds turn the eggs to expand the area of the limiting membrane that contacts the agent-producing cells (Yoshizaki and Saito, 2002).

Oviparous reptiles spawn eggs in a wide range of environments, from wet habitats to dry land. It is not clear how the water balance inside the egg is controlled and how calcium is taken up from the eggshell (Packard et al., 1984) or the environment. Most studies in this area have focused on the structure of the outer eggshell (Solomon and Baird, 1976; Packard and Packard, 1979; Packard, 1980; Packard et al., 1979, 1982, 1984; Kitimasak et al., 2003). The present study shows the structure and nature of the shell membranes in commercially available eggs of the Chinese soft-shelled turtle (*Pelodiscus sinensis*), and compares them with those in Japanese quail (*Coturnix japonica*) eggs.

Materials and Methods

Fertilized eggs of Chinese soft-shelled turtles were purchased from a dealer in Hamamatsu during July and August. These eggs were embedded in styrene foam boxes filled with wood shavings to which moisture was occasionally added, and incubated at 30C, as recommended by Tokita and Kuratani (2001). Development of the eggs measured in days of incubation and embryonic development at each day was confirmed according to the normal table of Tokita and Kuratani (2001).

After embryos and egg whites were removed, the shells and shell membranes were immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2. The isolated shell membranes were further fixed in the same solution for 3 h. The specimens were rinsed in the buffer, postfixed with 1% osmium tetroxide in the same buffer for 1 h, dehydrated in acetone, and embedded in epoxy resin for transmission electron microscopy. Thin sections were stained with uranyl acetate and lead citrate. For scanning electron microscopy, specimens were dehydrated in acetone, dried in a critical point apparatus, HCP-1 (Hitachi, Japan), coated with a layer of gold in an IB-3 ion coater (Eiko, Japan), and examined under a model S-4300 scanning electron microscope (Hitachi, Japan).

For the determination of water permeability, the shell membrane was treated as reported previously (Yoshizaki and Saito, 2002). Briefly, the shell membrane was removed from the emptied eggshell by treatment with 0.5 N HCl for 3 min. After being washed repeatedly with distilled water, the shell membrane was attached, with its limiting membrane facing inwards, to the lower end of a glass tube (5.5 mm in diameter) with a cyanoacrylate glue, AlonAlpha (Konishi Co., Japan). The glass tube was filled with 1.5 ml of distilled water and then covered at its upper end with a paraffin film in which a pinhole (0.5 mm in diameter) was made. These preparations were set in an incubator at 39C with 60% humidity for 24 h. The incubation temperature adopted was determined to compare the results with those for quails (Yoshizaki and Saito, 2002). The amount of water that evaporated through the membrane was determined by measuring the distance the meniscus moved.

Data were analyzed with two-way nested analysis of variance (ANOVA). Statements of significance were based on $P \leq 0.01$.

Results

Water permeability of the shell membrane

The water permeability of the shell membrane was measured based on the evaporation of distilled water through membranes from eggs incubated for 0, 6, 8, 23, 28, 31, and 54 days. The average water permeability was 5 nl/mm^2 per min.There were no significant differences in this parameter among membranes regardless of the incubation period ($F_{(6,6)}=5.54$; $P=0.028$) (Fig. 1). The difference was not significant, either, between membranes facing the embryo proper (embryonic side) and those facing the yolk (yolk side) ($F_{(1,6)}=0.735$; $P=0.424$) (Fig. 1).

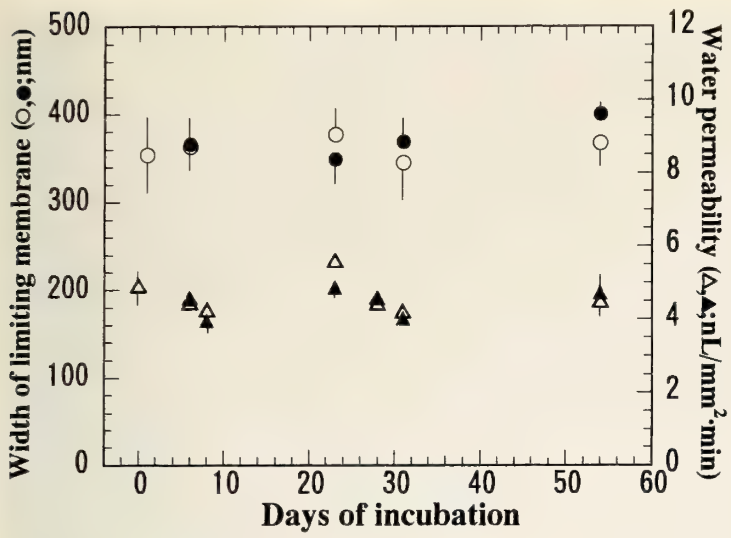

FIG. 1. The width of limiting membranes and the water permeability of shell membranes on the embryonic (open symbols) and yolk (closed symbols) sides in eggs of the Chinese soft-shelled turtle during incubation. Values are means of five experiments. Vertical bars denote ranges of SD.

Morphological observations of the shell membrane

The shell membrane which is located between the calcareous shell and the albumen had a thickness of about 100 μm (Fig. 2A). It was organized into an inner shell membrane, adjacent to the albumen, and an outer shell membrane, adjacent to the mineral layer. The main body of the shell membrane was a meshwork of intersecting fibers (Fig. 2B, C) consisting of two components in cross section (Fig. 3A, C), the medulla and cortex, which randomly run in the plane of the membrane. The fibers in the outer portion of the inner membrane fused via their cortex, thus forming a flat shape (Fig. 2B, 3B). At its interface with the egg white, the shell membrane was demarcated by a layer of dense material called the limiting membrane (Fig. 3C). We measured the thickness of the limiting membrane of eggs on days 1, 6, 23, 31, and 54 of incubation. The average thickness was 362 nm. No significant differences were observed in this measurement among the different incubation periods ($F_{(4,4)}=0.940$; $P=0.523$), nor between membranes of the embryonic side and the yolk side of individual eggs ($F_{(1,4)}=0.364$; $P=0.579$) (Fig. 1).

With careful observation, we could identify canals in the limiting membrane, which run perpendicularly or obliquely to the plane of the membrane (Fig. 3D). When observed from the inside with the scanning electron microscope, the membrane was seen to have many holes (Fig. 2D). The largest holes were 20 nm in diameter. This ultrastructural feature was observed in all eggs regardless of incubation period.

DISCUSSION

The thickness of the limiting membrane of quail eggs gradually decreased from 74 nm to 35 nm with development. Conversely, water permeability through the shell membrane increased from 4 to 5 nl/mm^2 per min, to 9 nl/mm^2 per min at 39C (Yoshizaki and Saito, 2002). These changes in the Aves are thought to satisfy the requirements for gas exchange and for the mobilization of calcium from the eggshell. In unturned eggs, both the decrease in the thickness of limiting membranes and the increase in water permeability are delayed on the yolk side. Egg turning, which is needed in most avian eggs for normal embryonic development to take place, is understood to expand the area of the limiting membrane in contact with the enzyme-producing cells, thus enabling the thickness of the membrane to be sufficiently and equally reduced over the whole surface (Yoshizaki and Saito, 2002).

The rigid-shelled eggs of chelonians, such as those of the Chinese soft-shelled turtle, provide a great deal of resistance to the movement of water into and out of eggs (Packard et al., 1979), and as a result, such eggs are virtually insensitive to variations in the hydric environment (Packard et al., 1982). However, water exchange through the shell membrane might occur inside the shell, since such turtles may use the shell as a source of calcium during embryogenesis (Packard et al., 1984; Ewert, 1985). The shell membrane of the turtle, despite having a limiting membrane that is more than four times thicker than that of the quail, exhibited a similar water permeability to that of early embryos of the quail.

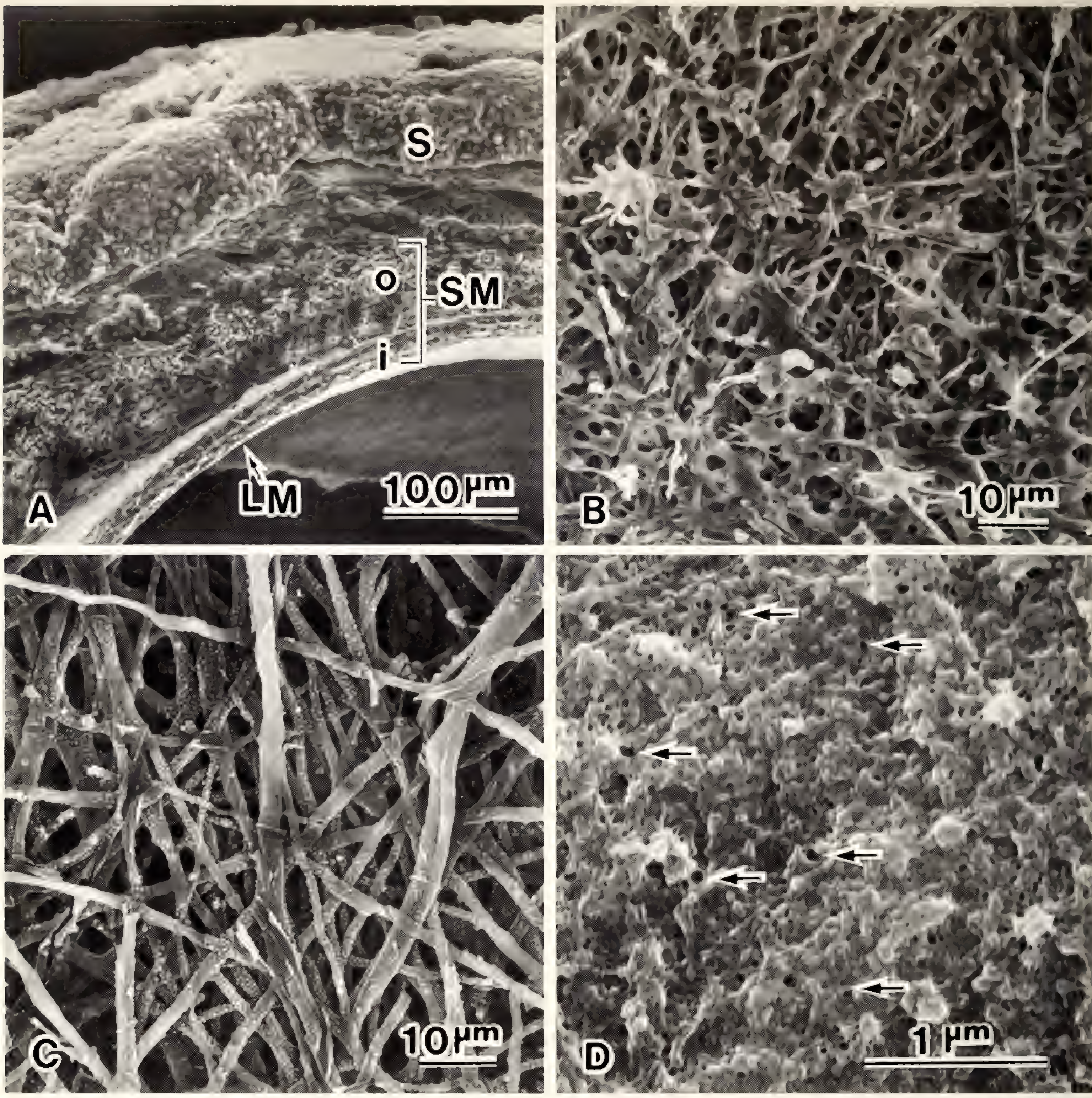

FIG. 2. Scanning electron micrographs of shell membranes from the Chinese soft-shelled turtle eggs on day 6 of incubation. (A) The shell membrane (SM) has a width of about 100 µm, and is organized into an inner shell membrane (i) and an outer shell membrane (o). S, shell. (B, C) Inner shell membrane. The shell membrane is a meshwork of fibers. In the outer portion of the membrane (B), the fibers fuse, forming a flat shape. (D) A scanning image of the inner surface of a limiting membrane (LM) shows many holes (arrows).

The canals which run perpendicularly through the limiting membrane may explain this water passage through such thick membranes in turtles. Although the shell membrane has a thickness of about 100 µm in the present species and about 260 µm in *Chitra chitra* (see Kitimasak et al. [2003]), it might be the limiting membrane that retains the water because the main body of the shell membrane is made up of roughly parallel, intertwining fibers.

The canals are apparently not present in the limiting membranes of quail eggs (Yoshizaki and Saito, 2002). Their absence may be explained by the contribution of another egg envelope, the peri-albumen layer, located on the outermost surface of the egg white (Sultana et al., 2003). The peri-albumen layer in an ovulating egg appears at a narrow ring-like

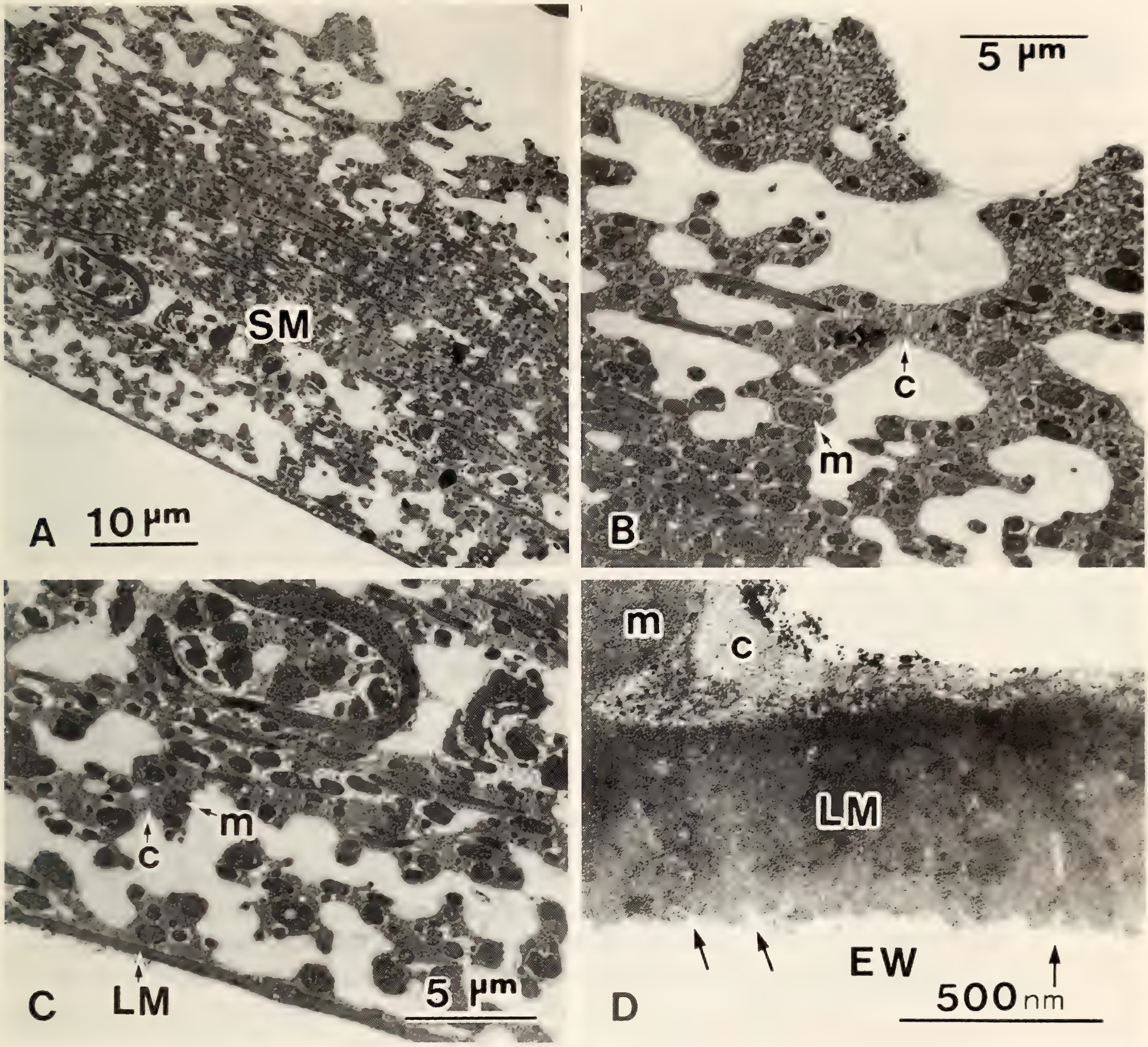

FIG. 3. Transmission electron micrographs of inner shell membranes from the Chinese soft-shelled turtle eggs on day 6 of incubation. (A, B) The fibers of shell membranes (SM) consist of two components, the medulla (m) and cortex (c). In the outer portion of the membrane, the fibers fuse to each other via their cortex. (C, D) At the interface with the egg white (EW), the shell membrane is demarcated by a layer of dense material called the limiting membrane (LM). There are small canals which run perpendicularly or obliquely to the plane of the membrane (arrows).

constriction of the magnum-isthmus junction of the oviduct, makes the outermost surface of the egg white smooth, and determines the shape of the egg white mass. Thus, the limiting membrane subsequently produced at the isthmus might be formed uniformly without any obstructions. In soft-shelled turtle eggs, no peri-albumen layer was observed between the egg white and limiting membrane (unpublished), and the magnum-isthmus junction is apparently absent in the oviduct (Girling, 2002). Then the materials for the limiting membrane might be polymerized on an irregular egg white surface, and as a result, canal-like structures may appear as a vestige of ciliary processes of the egg white.

Turtle eggs are not turned during embryogenesis, unlike avian eggs. Neither the thickness of the limiting membrane nor permeability of the shell membrane change, indicating an absence of machinery which affects the structure of the membranes. Hence, turtle eggs

maintain a minimal rate of gas-exchange and mineral uptake throughout embryogenesis (Feder et al., 1982). They therefore take 54 days of incubation to hatch out compared to 17 days for quail eggs, a big difference even considering the difference in incubation temperature.

In reptiles, some chelonians and most squamates produce flexible-shelled eggs which have little (chelonians) or no (squamates) calcareous layer. These eggs are more or less dependent on the hydric environment in terms of embryonic growth and hatching success (Packard et al., 1979, 1982). The ecological significance of water exchange in reptilian eggs has been documented by focusing on the structure of the outer eggshell (Solomon and Baird, 1976; Packard and Packard, 1979; Packard, 1980; Packard et al., 1979, 1982, 1984) and to a lesser extent, on the shell membrane (Packard, 1980; Feder et al., 1982), but not on the limiting membrane. The present study indicates the need to compare the structure of the limiting membrane for a total understanding of the reproductive strategy of the Reptilia, because all members of this group that lay eggs on land produce eggs with a limiting membrane to retain water.

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Molecular Phylogeny of the Genus *Acanthosaura* (Agamidae)

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Abstract: A 900 bp fragment of mitochondrial cytochrome b was sequenced from 63 specimens of the arboreal agamid lizard genus *Acanthosaura* from Vietnam, Laos, Myanmar (Burma), Thailand and Malaysia, representing all four currently recognized species. A hypothesis of maternal relationships was generated based on a maximum parsimony analysis of 44 different haplotypes. *Pseudocalotes* and *Calotes* were used as outgroup taxa. The genealogical analyses of the cytochrome b sequences recovered four lineages represented by *A. armata*, *A. capra*, *A. crucigera*, and *A. lepidogaster*. A fifth lineage consisted of one specimen from Ngoc Linh, Vietnam that was considered to be *A. lepidogaster*; however, recognizing it as such would render *A. lepidogaster* paraphyletic. *Acanthosaura crucigera* consisted of two clades. One of these clades contains cysteine in a portion of its cytochrome b, and is the sister group of all other species of *Acanthosaura*. In turn, the second clade of *A. crucigera* formed the sister group of *A. armata*, *A. capra*, and *A. lepidogaster.* A clade containing *A. armata*, *A. capra* and the sample from Ngoc Linh, Vietnam was the sister group to *A. lepidogaster.*

Key words: *Acanthosaura*; Agamidae; Vietnam; Thailand; Laos; Molecular phylogeny; Cytochrome b

INTRODUCTION

Acanthosaura Gray 1831 is a genus of arboreal agamids distributed in southern China, Vietnam, Thailand, western Malaysia, including Pulau Pinang and Pulau Tioman, Laos, Cambodia, Myanmar, and Indonesia (Sumatra and Anamba) (Manthey and Crossmann, 1997). These lizards are usually restricted to evergreen forests at elevations up to 1800 m, and prefer riparian habitats.

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The genus contains four species. The relatively most common species, *A. lepidogaster*, occurs in northern Thailand, Vietnam, Cambodia, Myanmar, Laos, and southern China (Yunnan, Guangxi, Guangdong, Fujian, Hainan). *A. capra* is known from Cambodia, Laos and Vietnam. *A. armata* is distributed in southern Thailand north to the Nakhon Si Thammarat Province, western Malaysia as well Palau Pinang and Pulau Tioman, and Indonesia (Sumatra). Finally, *A. crucigera* was recorded from Myanmar, Thailand, Central Vietnam (Annam), West Malayasia, and Cambodia (Boulenger, 1885; Smith, 1935; Taylor, 1963; Manthey and Grossmann, 1997). The genus has not undergone any recent taxonomic re-evaluation. The phylogenetic relationships of the species have never been evaluated.

The taxonomic status of the genus *Acanthosaura* is unclear. Some authors have treated it as a junior synonym of *Gonocephalus* (Smith, 1935); and others have referred some species to the genus *Japalura* (Boulenger, 1890). Several described species have been synonymized with *Acanthosaura lepidogaster* (Wermuth, 1967). Recently, most herpetologists have recognized *Acanthosaura* as containing the four species *armata*, *capra*, *crucigera*, and *lepidogaster* (Manthey and Schuster, 1992; Ananjeva, 1997). Although this association was partly supported by recent molecular data (Macey et al., 2000), the taxonomy of the genus and relationships among its species remain obscure. The problem derives from the great anatomical similarity of the taxa. Variability in the diagnostic characters, such as length of the nuchal and postorbital spines, and length of the diastema between nuchal and dorsal crests, causes much taxonomic confusion. The only species easily diagnosed is *A. capra*. It differs from the other species of *Acanthosaura* by having only one pair of postorbital spines and no nuchal spines.

Relatively few specimens of *Acanthosaura* (except *A. lepidogaster*) exist in museum collections because of a limited historical collecting. The paucity of specimens precludes statistically based analyses of morphological variation and relationships within this genus. Consequently, we pursued a molecular evaluation of the relationships of the matriarchic lineages among samples of *Acanthosaura*. Mitochondrial DNA (mtDNA) is commonly used in population and evolutionary systematics (Hillis et al., 1996). We chose cytochrome b (cytb) because of its applicability in relatively recent divergences (Wilson et al., 1985; Moritz et al., 1987; Harrison, 1989). Our investigation crosses the boundary between micro- and macroevolution because we investigated multiple individuals from many collecting sites, and all four recognized species.

Materials and Methods

Blood or liver tissues from 63 individuals representing all four currently recognized species were obtained from throughout the range of *Acanthosaura* (Fig. 1; Appendix). The agamids *Pseudocalotes brevipes* and *Calotes versicolor* were used as the outgroup taxa. Choice of outgroup taxa is based on phylogenetic relationships revealed by Macey et al. (2000).

FIG. 1. Map showing the distribution of the genus *Acanthosaura* and localities sampled in this study.

Laboratory protocols

DNA was extracted from liver and blood tissues following standard proteinase k and phenol chloroform protocols (Sambrook et al., 1989). A fragment of mtDNA containing most of cyt-b and tRNA^{threonine} was amplified from 1–6 μl aliquots of isolated DNA using the polymerase chain reaction (PCR). The light and heavy strand primers used were modified versions of those given by Kocher et al. (1989) (Table. 1). Amplification conditions were as follows: after an initial denaturation at 94 C for 300 s, 31 cycles followed with a denaturation at 94 C for 45 s, annealing at 42–45 C for 45 s, and extension at 70 C for 120 s. Cycle sequencing reactions used a two step program: 15 cycles followed with denaturation at 94 C for 45 s, annealing at 47–53 C for 45 s, extension at 70 C for 60 s, and 15 cycles of denaturation at 94 C for 45 s and extension at 60 C for 60 s. Three sequencing primers were used (the light strand primer mt-a, and two heavy strand primers, smt-f and mt-b2) to obtain sequences of both strands using an automatic sequencer (ALF Express). Sequencing was performed for 5–11 hr depending on the length of the fragment. Each sequence was verified by sequencing from heavy and light strand primers with a large overlap of the segments and from different PCR amplification products.

Phylogenetic analysis

Alignment of the cyt-b sequences was achieved manually. The base composition, transition/transversion ratio, genetic distances, and phylogenies were estimated using PAUP* (ver. 4.0b10; Swofford, 2000).

Phylogenetic relationships are generated by maximum parsimony (MP) (Siddall and Kluge, 1997; Kluge, 2002). All trees were calculated using PAUP*, and were constructed using heuristic searches with tree bisection-reconnection (TBR) branch swapping and saving all most parsimonious trees (MPTs). Addition-sequence replication was performed 1000 times holding 10 trees at each step. Only potentially phylogenetically informative sites were used in the analysis, and the starting tree was obtained by random stepwise addition. All characters were evaluated as unweighted. Constraint trees were constructed using MacClade (ver. 4.00; Maddison and Maddison, 2000).

Nodal consistency was assessed by decay analysis (Bremer, 1994) and nonparametric bootstrap analysis with 10,000 pseudoreplicates. Decay analysis was calculated using AutoDecay ver. 4.0 (Eriksson, 1998). For each bootstrap replicate, two heuristic searches were performed with closest addition of taxa.

A suite of preferred trees was obtained by using successive approximations weighting (Farris, 1969; Carpenter, 1994). Reweighting was based on the retention index (Farris, 1989) using the best fit (maximum value) and a base weight of 1. The data were reweighted until a consistent tree length was obtained. Following

TABLE 1. Primer sequences (5'–3') of cytochrome *b* used for amplifying DNA from agamid lizards of the genus *Acanthosaura*. Positions in the chicken genome corresponding to the 3' end positions of primers are given in parentheses. L and H are heavy-strand and light-strand DNA, respectively.

Primer	Sequence
smt A (L-14995)	5'-CAACATCTCAGCATGATGAAACTTCG-3'
mt A-new (L-14995)	5'-TCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3'
mt C (L-15311)	5'-GCAAGTCTTCTACCATGAGGACAAATATC-3'
mt E (H-15698)	5'-AATAGGAAGTATCATTCGGGTTTGT-3'
mt F-new (H-16060)	5'–AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG-3'
smt F (H-16060)	5'–TCAGTTTTTGGTTTACAAGACCAATG-3'
mt B2 (H-15298)	5'–GCCCAGAAkGATATTTGTCCTCA–3'

analysis, data weights were reset to 1, and the suite of trees was sorted for length. Consensus trees (strict and 50% majority rule) were constructed from the suite of equally most parsimonious trees.

RESULTS

A 900 bp fragment of cyt-b was obtained from most of the 63 samples of *Acanthosaura*. Multiple specimens represented most sampling localities. Some individuals had identical sequences. These were combined for analysis resulting in 44 different mtDNA haplotypes from *Acanthosaura*. Homologous sequences from *Calotes versicolor* and *Pseudocalotes brevipes* were obtained and used for the analyses. The sequences are deposited in GenBank (Accession Numbers AY572869–AY572930). Although the fragment was completely sequenced for most individuals, 716–720 bp were obtained for *A. capra* (A150–152, A159–160), 688–720 bp from *A. crucigera* (A69, A71, A153), and 684–704 bp from *A. lepidogaster* (A154–156, A158).

Authenticity of the mtDNA

None of the sequences contained premature stop codons, insertions, or deletions, and therefore do not appear to be nuclear copy pseudogenes. The amino acid sequences identified regions that are conserved among vertebrates. These regions corresponded to those of other animals (Howell, 1989; Irwin et al., 1991), suggesting that these sequences represent functional copies that encode a protein. The strong bias against guanine on the light strand found in all analyzed sequences (A=28.3–30.9%, C=30.1–32.0%, T=25.4–27.6%, G=12.0–14.3%) was characteristic of the mitochondrial genome (Kocher et al., 1989).

Substitutions and genetic variation

Among the 900 bp of cyt-b sequenced, 483 positions were variable and 381 were potentially phylogenetically informative. As expected, the most variable sites occurred in the third codon position (n=274), less variation occurred at the first position (n=127), and little variation was observed at the second position (n=82).

Absolute pairwise genetic distances of potentially phylogenetically informative sites only were calculated for all sequences of *Acanthosaura*. The amount of substitution varied from no site changes between individuals of a population to 118–219 sites between species. Between *Acanthosaura* and the outgroup taxa, differences occurred at 209–270 sites. The sequences of *Calotes* and *Pseudocalotes* differed at 252 sites. The transition/transversion ratio among the species of *Acanthosaura* varied from 12:1 to near 1:1, although the mean was 2.48:1. As expected, the transition/transversion ratio was higher among taxa near the terminal branches of the tree, and lower near the base of the tree.

Phylogenetic analysis

The unweighted maximum parsimony analysis of 1000 random additions of taxa resolved 562 trees, each with a length of 1296 steps, CI=0.48, RI=0.80, and RC=0.38. A 50% majority rule consensus tree is shown in Fig. 2. Most nodes were consistently resolved; 26 of 36 nodes had a BSP of 100%, seven ranged from 74–97%, and only three were supported by 52–55%. The data appeared to have significant structure.

Successive approximation weighting achieved a stable tree length after nine iterations. The maximum parsimony evaluation of the reweighted data resolved 231 trees. When character weights were reset to a value of one, tree lengths varied from 1296–1308 steps. Fifty-four trees had the same length as those obtained in the unweighted analysis; the strict consensus and the 50% majority rule consensus trees based on these trees were identical to each other, and to the 50% majority rule consensus tree based on the unweighted data (Fig. 2), except that the node uniting *A. lepidogaster* A55–56 with the clade (A8, A58, A60, A59) collapsed. Weighting first and second codon positions twice as much as the

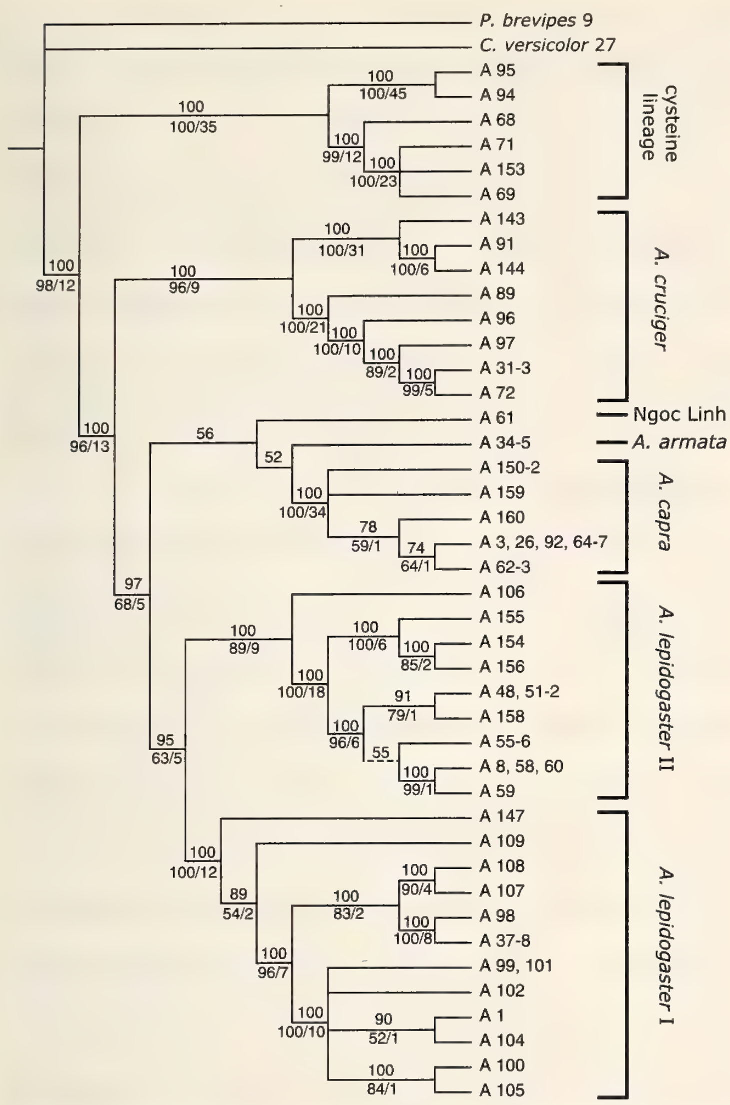

FIG. 2. A majority-rule consensus tree of 562 maximum parsimony trees depicting the maternal lineages among populations of lizards of the genus *Acanthosaura* derived from the analysis of cytochrome b sequence data. Numbers above the line are the consensus values and those below are decay values and bootstrap proportions. The dashed line connecting specimens within a clade of *A. lepidogaster* represents the single collapsed node obtained from successive approximations weighting, and from weighting first and second codon positions twice as much as the third. Specimen numbers refer to individuals in the Materials and Methods section of this paper.

third obtained the identical result of 54 most parsimonious trees and the same consensus trees.

Multiple individuals from the same sampling locality always clustered together, reflecting a shared historical maternal lineage. As expected, there was a high degree of concordance between geography and maternal history.

Within *Acanthosaura*, several maternal lineages could be clearly defined. Four lineages represented *A. crucigera*, *A. lepidogaster*, *A. armata*, and *A. capra*. A fifth lineage consisted of *A. lepidogaster* from Ngoc Linh, Vietnam (specimen A61). This sample from the Central Highlands made *A. lepidogaster* paraphyletic. A sixth lineage was formed by paraphyletic *A. "crucigera"* (Fig. 2). One lineage of *A. crucigera* (A68–69, A71, A94–95, and A 153) was the only group to have the amino acid cysteine in a portion of its cyt-b; this "cysteine lineage" is the sister group of all other *Acanthosaura*. The other lineage of *A. crucigera* is, in turn, the sister group of *A. lepidogaster*, *A. capra* and *A. armata* (Fig. 2).

The decay analysis found substantial support for nodes at the base of the tree. For example, 35 steps supported monophyly of the cysteine group, and 13 additional steps were required to break the monophyly of *Acanthosaura* exclusive of the cysteine group. Whereas the base of the tree exhibited great stability, more terminal groups that consisted of multiple specimens from nearby collecting sites did not receive much support.

High bootstrap proportions supported many nodes. These included all nodes resolved within the cysteine lineage of *A. crucigera*, the monophyly of *A. capra*, and the two groups of *A. lepidogaster.* Monophyly of *A. capra*, and the cysteine lineage was supported by a BSP of 100% (Fig. 2). A monophyletic *A. crucigera* (exclusive of the cysteine lineage) was supported by a BSP of 96%. A monophyletic *A. lepidogaster*, exclusive of A61, was supported by a BSP of 63%, and both maternal lineages of *A. lepidogaster* received high BSP supports of 100% and 89% (Fig. 2). Both "A61" and *A. armata* were each represented by a single unique sequence. Thus, their monophyly cannot be evaluated.

Acanthosaura lepidogaster, exclusive of A61, consists of two distinct subgroups. One, "*lepidogaster* I", includes specimens from Laos, north-central Vietnam, and Thailand. The second subgroup, "*lepidogaster* II", includes specimens from northern and northeastern Vietnam. The maternal genealogy resulted in limited geographic mixing. One

specimen of *A. lepidogaster* (A106) from Bolikhamxay Province, Laos (Fig. 1) consistently clusters with "*lepidogaster* II" (Fig. 2).

A single specimen of *A. lepidogaster*, A61, usually maintained its association with the clade of *A. armata* and *A. capra*. Specimen A61 did not cluster with other *A. lepidogaster* in any of the MPTs, even when it was combined with *A. armata*. However, a monophyletic lineage of *A. lepidogaster*, including A61 and excluding *A. armata*, requires only three additional steps on the MPT. This reduces to two additional steps if A61 and *A. armata* are a clade that forms the sister group to *A. lepidogaster*.

Acanthosaura crucigera, apart from the cysteine lineage, is clearly monophyletic. The genetically most distinctive population evaluated is from northern Myanmar (Bago Yoma, A91 and A143). Forcing *A. crucigera* to be a monophyletic species, while allowing for all other possible arrangements to occur on the tree, requires nine additional steps on the MPT.

Discussion

Phylogenetic relationships within the genus Acanthosaura

All maximum parsimony evaluations resolved extremely similar branching patterns. Macey et al. (2000) based on an analysis of sequences from several mtDNA genes, including ND1, ND2, COI and eight intervening tRNA genes, found that the two species of *Acanthosaura* in their investigation shared a common ancestor. Their survey of taxa was not sufficient to document the monophyly of all species of *Acanthosaura*. Unfortunately, the diversity of outgroup species in our dataset was insufficient to cladistically test this hypothesis. However, given the observed distances among our ingroup and outgroup taxa, we are very confident in the monophyly of *Acanthosaura*.

We consistently resolved a single ancestral maternal lineage for the four, long-recognized species and at least two not previously known. A single specimen from Ngoc Linh, Vietnam that strongly resembles *A. lepidogaster*, A61, represents one newly discovered lineage. The sixth, the "cysteine lineage," is the sister group of all other *Acanthosaura*. Conservatively, each of the six lineages represents at least one species. Support for monophyly of the six lineages was considerable. High BSPs supported the four lineages represented by multiple specimens. In addition, each of the six major lineages was supported by long branch lengths on the trees.

The cladogenic pattern among species in the "*crucigera*" lineage reflect a classic pattern of dispersal (Murphy and Aguirre-Léon, 2002), in this instance southward. The oldest cladogenic event isolated maternal lineages in northern Myanmar (A91, A1443–144, Bog Yoma) and more recent events occurred further south.

The extent of sequence divergence within *A. lepidogaster* forms an interesting problem for future investigation. Specimen A61, from Ngok Linh Mountain, Vietnam, did not cluster with other specimens of this species. Although geographically closer to some other specimens, it was very divergent in its cyt-b sequences (ca. 16% divergence, or an average of 144 nucleotide sites). Certainly, percent divergence cannot be used as an objective arbitrator for defining species (Frost and Hillis, 1990; Wake and Schneider, 1998). However, this high level of sequence divergence is indicative of speciation. Equally interesting, the two most divergent clades of *A. lepidogaster* exclusive of A61 also differ substantially. The average divergence of 12% generally exceeds that known to occur among individuals of other vertebrate species. This suggest that either two additional cryptic species have been combined in the name *A. lepidogaster*, or these lizards are a goldmine for future studies of evolutionary genetics. Sampling in the contact zone of the two haplotypes is critical.

The *A. capra* and *A. armata* lineages are well defined by the sequence data. They differ from one another at an average of 16% of the total nucleotide sites. The absence of clear cladistic relationships among samples of *A.*

capra reflects restricted sample sites—all 15 specimens are from two adjacent localities.

Taxonomic implications

The cysteine lineage includes *A. "crucigera"* from Krong Pa, Vietnam (A68), Cat Tien National Park, Vietnam (A69, 71 and 153), and pet trade specimens reputed to be from Myanmar (A94 and A95). The cysteine lineage can be diagnosed from other species of *Acanthosaura* using molecular characters; for example, it differs from other species by more than 20% of the amino acids in the sequenced fragment. Monophyly and the ability to diagnose are required operational criteria for recognition of species under any phylogenetic based species concept (e.g., Cracraft, 1989; Nixon and Wheeler, 1990; Brooks and McLennan, 1999, 2002). Our preliminary evaluations reveal that morphological differences separate the specimens of the two major "cysteine" subgroups from other species of *Acanthosaura*. Furthermore, the two specimens of *Acanthosaura* putatively from Myanmar cannot be morphologically associated with any known species. The cysteine lineage could consist of at least three species, considering the extent of divergence that occurs within Vietnam alone.

Although specimen "A61" from Ngoc Linh, Vietnam was initially identified as *A. lepidogaster*, this determination is now questionable. In most trees based on equally weighted data, it formed the sister group to *A. armata* plus *A. capra* (Fig. 2). These cladogenic resolutions require recognizing this population as different from *A. lepidogaster*. In support of this conclusion, some morphological characteristics separate A61 from other species of *Acanthosaura*. However, additional specimens from Ngoc Linh are desirable before describing the species. This finding is not particularly surprising given the high level of endemism that characterizes the fauna of primary forests of the Annam Mountains, including Ngoc Linh. (Darevsky and Orlov, 1994, 1997).

The two genetically distinct maternal lineages of *A. lepidogaster* are not diagnosable morphologically. Even though the maternal lineages are distinct, they might not be separate species. Gene flow may be ongoing where the two lineages come in contact. The extent of mtDNA divergence does not necessarily indicate an absence of gene flow. For example, in the lizard genus *Urosaurus*, historical lineages in Baja California, Mexico differ by 13% divergence in cyt-b (Johan Lindell, personal communication), and yet a cladistic allozymic study infers ongoing gene flow among the maternal lineages (Aguirre-Léon et al., 1999). Indeed, in the genetically best-studied geographic region, the central region of the peninsula of Baja California, Mexico, multiple species average 7–9% divergence in mtDNA without any indication of the absence of gene flow (Murphy and Aguirre-Léon, 2002; Riddle et al., 2000). Consequently, in the absence of morphological differentiation, nuclear gene analysis is required to document the presence of multiple cryptic species of *A. lepidogaster*. On a broader scale, our evaluation of mitochondrial cyt-b sequences revealed that at least seven lineages of *Acanthosaura* could be considered as discrete species, including two species within the cysteine lineage. Detailed morphological work or nuclear DNA data is needed to independently corroborate the hypothesis that theses lineages represent separate species.

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APPENDIX

List of vouchers used in this study and GenBank accession numbers of sequences. ZISP=Zoological Institute, St. Petersburg, Russia; ROM=Royal Ontario Museum, Toronto, Canada; CAS=California Academy of Science, San Francisco, USA; FMNH=The Field Museum, Chicago, USA; HLMD=Hessisches Landesmuseum, Darmstadt, Germany; PCUM=private collection of Ulrich Manthey, Berlin, Germany; PCUJ=private collection of Ulrich Joger; IRSNB=Royal Belgian Institute of Natural Sciences (Institut Royal des Sciences Naturelles de Belgique).

Acanthosaura armata

A34, A35-PCUM, Pulau Pinang (=Penang), Penang State, West Malaysia: 5°54'N, 100°12'E.

Acanthosaura capra

A3-ROM32160, A26-ROM32167, A64-ROM 31984, A65-ROM32154, A66-ROM32155, A67-ROM32160, A92-ROM31983, A150-ROM32161, A151-ROM32152, A152-ROM 32162, A159-ROM32143, A160-ROM32166, Krong Pa, Gia Lai Province, Vietnam: 14°20'29"N, 108°28'40"E.; A62-30627, A63-ROM30628, Tram Lap, Gia Lai Province, Vietnam: 14°26'24"N, 108°22'59"E.

Acanthosaura crucigera

A31, A32, A33-PCUM, Khao Lak, District (Amphoe) Takua Pa, Province Phang Nga, Thailand: 8°55' 8°65"N, 98°14' 98°45" E. A72-IRSNB15141, Phang Nga Wildlife Breeding Center, Tabon Tahk Daed, Thai Muang District, Phang Nga Province, Southern Thailand: 8°72'N, 98°27'E. A89-Kaeng Krachan, Kao Yoi Districts, Phetchaburi Province, Central Thailand: 13°99'N, 1002°25'E (tissue sample and photo from Peter P. Van Dijk). A91-CAS206626, A143-CAS208426, Bago Division, Bago Yoma, E. Myanmar: 18°52'59"N, 95°52'44.9"; A144-CAS206626, Bago Division, Bago Yoma, E. Myanmar: 18°81'67"N, 95°08' 50,4"; A96, A97-per trade, Malaysia.

Acanthosaura lepidogaster

A1-ROM26328, Khe Moi River, 24 km (by road) W of Con Cuong Village, Nghe An Province, Vietnam: 18°56'18"N, 104°53'01"E; A8-ROM31954, A58-ROM31957, A59-ROM 31960, A60-ROM35038, Chi Linh, Hia Duong Province, Vietnam: 21°12'44"N, 106°28'39"E; A-37-ZISP20753-1, A38-ZISP20753-2, Northern Annam, Vu Quang, Ha Tinh Province, Vietnam: 18°16'N, 105°15'E; A48-ROM30503, A51-ROM30720, A52-ROM30694. A158-ROM30693, Tam Dao, Vinh Phu Province, Vietnam: 21°27'25"N, 105°38'48"E; A55-ROM36073, A56-ROM36075, Quang Thanh, Cao Bang Province, Vietnam: 22°37'43"N, 105°54'46"E; A98-FMNH255481, Boualapha District, Khammouane Province, Laos: 17°17'N, 105°41'E; A99-FMNH255582, A101-FMNH 255583, A104-FMNH255581, Nghe An, Con Cuong District, Vietnam: 18°58'N, 104°48'E; A100-FMNH255585, A102-FMNH255587, A105-FMNH255584, Nghe An, Tuong Duong District, Vietnam: 19°03'N, 104°37'E; A103-FMNH255490, A109-FMNH255489, Vieng Tong District, Hauphan Province, Laos: 20°14'N, 103°12'E; A106-FMNH255491, Thaphabat District, Bolikhamxay Province, Laos: 18°27'N, 103°10'E; A107-FMNH255488, A108-FMNH 255487, Nakai District, Khammouane Province, Laos: 17°53'N, 104°55'E; A147-PCUM, Khao-Yoi, Thailand: 13°22'N", 99°81'40"E; A154-ROM38117, vicinity of Sa Pa, ~4 km W of Sa Pa Village., on tributary of Golden River (=Suoi Vang), Lao Cai Province, Vietnam: 22°18'59"N, 103°49'16"E.; A155-ROM38115, A156-ROM38116—vicinity of Sa Pa, ~5 km SW of Sa Pa Village., Lao Cai Province, Vietnam: 22°18'56"N, 103°49'35"E.

Acanthosaura sp.

A61-ROM37082, Ngok Linh, Kon Tum Province, Vietnam: 15°05'08"N, 107°55'42"E.

A153-ROM42241, A71-ROM42240, A69-ROM37083, Dong Nai, Cat Tien National Park, Dong Nai Province, Vietnam: 11°25'23"N, 107°25'42"E.

A68-ROM31985, Krong Pa, Gia Lai Provonce, Vietnam: 14°20'29"N, 108°28'40"E.

A94, A95-HLMD-RA2969-26970. per trade, Myanmar.

Calotes versicolor

A27-HLDM 57—per trade, Vietnam

Pseudocalotes brevipes

A9-ROM 30515, Pac Ban, Na Hang Nature Reserve, Tuyen Quang Province, Vietnam: 22°25'05"N, 105°38'05"

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A New Species of *Dixonius* (Squamata: Gekkonidae) from Kanchanaburi Province, Western Thailand

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Abstract: A new species of the endemic Southeast Asian gekkonid lizard genus *Dixonius* Bauer et al., 1997 is described on the basis of material from Kanchanaburi Province in western Thailand. It is characterized by a distinctive orange tail and a prominent dark stripe from the snout to at least the level of the ear. It occurs sympatrically with its larger and more widely distributed congener, *D. siamensis*.

Key words: *Dixonius*; Gekkonidae; New species; Kanchanaburi; Thailand

INTRODUCTION

The gekkonid genus *Dixonius* Bauer et al., 1997 was erected to accommodate Southeast Asian leaf-toed geckos previously assigned to the polyphyletic and nearly cosmopolitan *Phyllodactylus*. The phylogenetic affinities of *Dixonius* to other gekkonids remain uncertain, but it does not appear to be especially closely related to other clades of leaf-toed geckos that have been identified (Bauer et al., 1997). The possible generic distinctness of the group was first noted by Annandale (1905b), who considered the presence of precloacal pores as highly distinctive within *Phyllodactylus*. Dixon (1964), subsequently noted that *siamensis* exhibited a reduced manual phalangeal formula of 2:3:4:4:3. Russell (1972) demonstrated that there was in fact no phalangeal loss in digit IV of the manus, but identified a unique reduction in size of phalanx II of this digit. Bauer et al. (1997) subsequently diagnosed *Dixonius* relative to other leaf-toed geckos on the basis of these precloacal pore and digital characters, as well as the tuberculate condition of the dorsum and the proximal bifurcation of the hypoischium.

Two species of *Dixonius* have generally been recognized by recent reviewers (Wermuth, 1965; Kluge, 1991, 1993, 2001; Bauer

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et al., 1997; Rösler, 2000). The larger and more widespread form, *D. siamensis* (Boulenger, 1898) was described from specimens from "Dung Phya Fai, Siam" [=Dong Paya Fai Mountains, Sara Buri and Nakhon Ratchasima Provinces, Thailand]. Two additional leaf-toed geckos described early in the 20th Century are currently placed in the synonymy of *D. siamensis*. Mocquard (1904) described *P. paviei* from "Vatana (Siam)", but provided no differential diagnosis with respect to *P. siamensis*, as he was apparently unaware of Boulenger's (1898) earlier description. Annandale (1905a), although cognizant of *Phyllodactylus siamensis*, did not know of *P. paviei* when he described *P. burmanicus* from "Tavoy" (Dawei, Taninthayi State, Myanmar). He proposed several diagnostic differences between his form and *P. siamensis*, including smaller dorsal tubercles and a smaller number of subdigital lamellae. However, the accumulation of many additional specimens has revealed that the purportedly diagnostic features of both *P. burmanicus* and *P. paviei* fall within the range of variation of *D. siamensis*.

A largely patternless form first noted by Smith (1935), however, was subsequently described by Taylor (1962: 215) as *Phyllodactylus melanostictus*, with its type locality at "Mauk Lek Road-Camp (Friendship Highway) Sara Buri" Thailand (corrected to Muak Lek by Taylor, 1962: 218; both spellings were also given by Taylor, 1963). This taxon differs from *D. siamensis* chiefly in its largely patternless dorsum and possession of a distinct dark line from the snout to the shoulder and thence along the flank to the tail. In addition, its specific distinctness is supported by its occurrence in sympatry with *D. siamensis* in Sara Buri and Nakhon Ratchasima provinces in central Thailand (Taylor, 1962, 1963; Grossmann and Ulber, 1990; Chan-ard et al., 1999). This form has also been reported from Ma Da, Vietnam (Bobrov, 1992), although whether the *D. melanostictus* is continuously distributed in the intervening areas or the Vietnamese population represents a disjunct population, or a similar but distinct species remains to be investigated.

Although *Dixonius siamensis* is best known from Thailand, where it occurs from Songkhla (7°N), south of the Isthmus of Kra (Taylor, 1963), north to at least Chiang Mai (19°N) (Grossmann et al., 1996; Manthey and Grossmann, 1997), its range extends from southern Myanmar (Annandale, 1905a, b) to the Lao Peoples Democratic Republic (Stuart, 1999) and Vietnam (Smith, 1935; Bourret, 1939; Szczerbak and Nekrasova, 1994). Its occurrence in Cambodia has not been verified, but the proximity of both Thai and Vietnamese localities to the borders of Cambodia (Szczerbak and Nekrasova, 1994; Grossmann et al., 1996) suggests that it is likely to range across the width of this country as well. This broad distribution, along with obvious geographic variation in color pattern (Taylor, 1963) raises the possibility that *D. siamensis*, as presently construed, may actually represent a complex of similar species.

This hypothesis is supported by Ota et al. (2001), who have demonstrated that a minimum of two chromosome forms (2n=40 and 2n=42) exist among Thai populations of *D. siamensis*, with a female from one population (from Mae Yom, northern Thailand) also exhibiting a heteromorphism indicative of a ZW sex chromosome system. Such karyotypic diversity strongly suggests that more than one evolutionary unit is presently included under *D. siamensis* (Ota et al., 2001). This also raises the possibility that one or both of the two names currently included in the synonymy of *D. siamensis* may be applicable to a valid species. Such a hypothesis cannot be adequately investigated without fine scale sampling of material for both morphological and molecular analysis from throughout the range of *Dixonius*, including each of the type localities. While such a revision is not yet possible, we here identify and describe a distinctive form of *Dixonius*, first collected in 1992 by Gernot Vogel from Kanchanaburi Province, western Thailand where it occurs in sympatry with typical *D. siamensis*.

Materials and Methods

The following measurements were taken with Brown and Sharpe Digit-cal Plus digital calipers (to the nearest 0.01 mm): snout-vent length (SVL; from tip of snout to vent), head length (HeadL; distance between retroarticular process of jaw and snout-tip), head width (HeadW; maximum width of head), head height (HeadH; maximum height of head, from occiput to underside of jaws), orbital diameter (OrbD; greatest diameter of orbit), nares to eye distance (NarEye; distance between anteriormost point of eye and nostril), snout to eye distance (SnEye; distance between anteriormost point of eye and tip of snout), eye to ear distance (EyeEar; distance from anterior edge of ear opening to posterior corner of eye), ear length (EarL; longest dimension of ear), internarial distance (Internar; distance between nares), and interorbital distance (Interorb; shortest distance between left and right supraciliary scale rows), trunk length (TrunkL; distance from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion), forearm length (ForeaL; from base of palm to elbow), crus length (CrusL; from base of heel to knee), tail length (TailL; from vent to tip of tail), tail width (TailW; measured at widest point of tail). Scale counts and external observations of morphology were made using a Nikon SMZ-10 dissecting microscope. Radiographic observations were made using a Faxitron enclosed radiographic unit.

Comparisons were made with museum material in the collections of the California Academy of Sciences (CAS), Field Museum of Natural History (FMNH), and Institut Royal des Sciences Naturelles de Belgique (IRSNB) (see Appendix), as well as original published descriptions and descriptions provided in broader faunal treatments (e.g., Smith, 1935; Taylor, 1963).

FIG. 1. Holotype of *Dixonius hangseesom*, sp. nov. (CUMZ R 2003.58) from Sai Yok, Kanchanaburi Province, Thailand. Bar equals 5 mm. Photo by Aaron M. Bauer.

Dixonius hangseesom, sp. nov.
Figs. 1–6

Holotype

Chulalongkorn University Museum of Zoology (CUMZ) R 2003.58 (Fig. 1); Thailand, Kanchanaburi Province, Sai Yok District, near Ban Tha Sao (Sai Yok Noi Waterfall), 14°06'N 99°25'E; Coll. M. Sumontha, 6 September 2002.

Paratypes

CUMZ R 2003.57, CUMZ R 2003.60, CUMZ R 2003.6, Institut Royal des Sciences Naturelles de Belgique (IRSNB) 2587; same data as holotype. Zoologisches Museum

FIG. 2. Juvenile captive specimen *Dixonius hangseesom*, sp. nov. (live collection of W. Grossmann) illustrating the drab tail coloration of young specimens. Photo by Wolfgang Grossmann.

Berlin (ZMB) 65437; Thailand, Kanchanaburi Province, Sai Yok National Park, Coll. G. Vogel, 28 September 1992.

Diagnosis

Dixonius hangseesom may be distinguished from non-congeneric leaf-toed geckos on the basis of the possession of precloacal pores by males and by the unique configuration of the phalanges of the manus. It may be diagnosed from *D. siamensis* by its smaller size (maximum 42 mm vs 57 mm; Manthey and Grossmann, 1997) and presence of a bold dark stripe through the eye, cross-banded or reticulate dorsal pattern, and an orange-colored tail (in life). It is also distinguished by its more irregular (with dorsal tubercles sometimes scattered on flanks and often with keels oriented obliquely to body axis) and partly imbricating dorsal scalation (vs smaller, regular, non-overlapping, more conical tubercles) and broader terminal scansors (approximately twice mid-digital toe width vs 1.5 times mid-digital toe width). These features would also diagnose the new species from *D. paviei* and *D. burmanicus*, should future research revalidate these taxa.

FIG. 3. Living specimen of adult male *Dixonius hangseesom*, sp. nov. from Sai Yok, Kanchanaburi Province, Thailand. Note the bright orange tail, crossbanded dorsal pattern and dark markings on sides of head. Photo by Montri Sumontha.

FIG. 4. Male paratype (ZMB 65437) of *Dixonius hangseesom*, sp. nov. shortly after capture as a 33 mm SVL subadult-adult. Compare tail coloration and dorsal pattern with Fig. 5. Photo by Wolfgang Grossmann.

FIG. 5. Male paratype (ZMB 65437) of *Dixonius hangseesom*, sp. nov. after seven years and three months in captivity. Note the fusion of dorsal dark markings and the drab tail in comparison to the same specimen earlier in life (Fig. 4). Photo by Wolfgang Grossmann.

Dixonius hangseesom shares the dark eye stripe with *D. melanostictus* but unlike this form has a dorsal pattern with alternating light and dark markings (vs an essentially unpatterned dorsum, or pale stripes or longitudinal series of light spots), a greater number of midbody scale rows (12–14 rows of tubercles and 22–26 ventrals vs 10–11 rows of tubercles and 22 ventrals; Taylor, 1963), and smaller body size (maximum 42 mm SVL vs 50 mm SVL).

Description of holotype

Adult male, snout-vent length 36.92 mm. Head relatively long (HeadL/SVL ratio 0.29), wide (HeadW/HeadL ratio 0.60), not markedly depressed (HeadH/HL ratio 0.40), distinct from slender neck. Lores and interorbital region weakly inflated, canthus rostralis relatively prominent. Snout moderately short (SnEye/HeadL ratio 0.37), rounded; longer than eye diameter (OrbD/SnEye ratio 0.69); scales on snout and forehead small, hexagonal to rounded, flattened, with smooth or slightly rugose surface and a low median keel, some conical; scales on snout larger than those on occipital region. Eye moderately large (OrbD/HeadL ratio 0.26); pupil vertical with crenelated margins; supraciliaries short, without spines. Ear opening oval, obliquely oriented, relatively large (EarL/HeadL ratio 0.08); eye to ear distance somewhat greater than diameter of eyes. Rostral approximately two-thirds deep (1.07 mm) as wide (1.63 mm), incompletely divided dorsally by a straight rostral groove; two somewhat enlarged supranasals in broad contact anteriorly, separated by a single, small internasal posteriorly; rostral in contact with supralabial I, supranasals, and internasal; nostrils round, each surrounded by supranasal, rostral, first supralabial, and two enlarged postnasals; one row of small scales separate orbit from supralabials. Mental triangular, wider (1.97 mm) than deep (1.51 mm); two pairs of enlarged postmentals, anteriormost approximately 2.5 times larger than posterior, posterior approximately 4–6 times larger than adjacent throat scales; each anterior postmental bordered anteriorly by mental, medially by other anterior postmental, anterolaterally by first infralabial, posterolaterally by second postmental; the pair collectively bordered posteromedially by a row of three throat scales; posterior postmentals each bordered posteriorly by series of 4–5 granules. Supralabials to midorbital position 6; enlarged supralabials to angle of jaws 8; infralabials 6 enlarged and two very small; interorbital scale rows across narrowest point of frontal bone 10.

Body slender, elongate (TrunkL/SVL ratio 0.43) with no ventrolateral folds. Dorsal scales strongly heterogeneous: small, irregular, flattened to conical scales interspersed among large (4–6 times size of adjacent scales), strongly

keeled subimbricate tubercles arranged in 12 more-or-less regular longitudinal rows extending on to tail; paravertebral tubercles especially well-developed; occasional tubercles with keels oriented obliquely to the body axis; flanks covered with irregular smooth scales. Ventral scales comparable in size to dorsal tubercles, smooth, imbricate; free margins rounded and distinctly ctenate; increasing in size from throat to chest to abdomen, somewhat smaller in precloacal region; midbody scale rows across belly to lowest rows of tubercles, 26; gular region with relatively homogeneous, granular scales. Eight precloacal pores in continuous series; pore-bearing scales not enlarged relative to adjacent scale rows; scales in row immediately posterior to pore-bearing row 2–3 times size of other scales of cloacal region. No femoral pores or enlarged femoral scales. Scales on palm and sole small, smooth, rounded to oval; scalation on dorsal aspects of hind limbs with enlarged, subimbricate, keeled tubercles on thigh and conical (or keeled) scales on shanks.

Fore and hindlimbs short, slender (ForeaL/SVL ratio 0.13; CrusL/SVL ratio 0.16); digits slender, dilated distally, all bearing robust, slightly recurved claws; basal subdigital lamellae narrow, without scansorial surfaces (6–8–10–9–9 manus; 6–9–11–13–13 pes); setae-bearing lamellae restricted to enlarged, distal, "leaf-like" scansors; interdigital webbing absent. Relative length of digits (manus): III>IV~II>V>I; (pes): IV>III>V>II>I.

Mostly original (terminal 19.97 mm regenerated) tail long, slender, tapering to tip; longer than snout-vent length (TailL/SVL ratio 1.34); whorls of keeled scales on dorsum of basal portion of tail, lateral and distal scales lacking well-developed keels; small, irregular scales separating keeled tubercles in some places; ventral scales enlarged into transverse plates. Regenerated portion of tail with more-or-less uniform, smooth, flattened, subimbricate dorsal scales; ventral scales as in original, but somewhat irregular in outline. Series of two small, smooth, raised postcloacal spurs on each side of tailbase.

Osteology

Parietal bones paired; stapes imperforate. Phalangeal formulae 2–3–4–5–3 for manus and 2–3–4–5–4 for pes. Presacral vertebrae 26, including 3 anterior cervical (without ribs), one lumbar, and 2 sacral vertebrae; 5 pygal and 7.5 post pygal caudal vertebrae to point of regeneration in Holotype and IRSNB 2587 (21.5 post-pygal vertebrae in CUMZ R 2003.6; 10.5 in ZMB 65437; 9.5 in CUMZ R 2003.60; 3.5 in CUMZ R 2003.57). Holotype and male paratypes with one pair of crescentic cloacal bones. Endolymphatic sacs enlarged extracranially in the two female paratypes, but not in males. Fusion of epiphyseal plates evident in all specimens except CUMZ R 2003.60 (partly fused).

Color in preservative

Boldly patterned, especially on head and anterior trunk, with contrasting cream and mid- to dark brown markings. Head with a series of concentric alternating light and dark arcs behind orbits; thick dark band from nostril, through orbit and above ear, fusing with dorsal dark brown markings on side of neck, this stripe bordered above by a narrower white stripe extending to level of ear; labial scales predominantly brown, white at sutures. Trunk predominantly brown with irregular cream cross bands, becoming less sharply defined on flanks and over sacrum. Limbs mottled. Tail pale brown with specklings of darker pigment; a single light band at junction of original with regenerate. Venter cream with isolated dark flecks on almost all scales, very faint along ventral midline, darker under limbs and tail and at flank margins.

Color in life

(Based on paratype ZMB 65437, captive specimens, and photographs of specimens in the wild). In life (Figs. 2–6) the dorsal base color is beige to grayish to yellowish tan with dark brown markings. A series of scattered whitish scales extending along dorsolateral margins of body from posterior margin of white supra-auricular stripe to tail base (not

evident in preserved specimens). There is a marked ontogenetic change in coloration. In juveniles (Fig. 2) the dark markings on the body may be more scattered than in adults. The tail in juveniles is also less brightly colored, being mid-brown with alternating beige to pale orange bands or spots. In older subadults and adults the tail takes on a distinctly orange base color that contrasts with that of the body. The tail may bear more-or-less regular lighter, yellowish bands with dark brown margins (Fig. 3), or may be a more uniform orange-brown (Fig. 4). In older adults there is maximal fusion of the dark dorsal markings and the tail darkens from orange to mid-brown (Fig. 5).

Variation

Comparative mensural data for the holotype and paratypes are presented in Table 1. The paratypes similar to holotype in most respects except as noted. CUMZ R 2003.6 with digits IV–V of right manus and digit III of left manus missing, digits II–IV of right pes missing, and digit V of same foot damaged. Female paratypes lack precloacal pores and cloacal bones. Precloacal pores in continuous series of seven (CUMZ R 2003.6) or eight (ZMB 65437), or with a single poreless scale separating left and right series of three pored scales (IRSNB 2587) in male paratypes. Dorsal tubercle rows 12–14, ventral scale rows 22–26, scattered tubercles present on flanks in ZMB 65437 (which possesses the most heterogeneous scalation of the type series). Dorsal coloration similar to holotype but darker brown and pattern more reticulate in ZMB 65437 (see also Fig. 5), paler and markings more regularly transversely arranged in remaining paratypes (see also Figs. 3, 6). Venter of ZMB 65437 moderately heavily pigmented, yielding a dusky appearance. Ontogenetic variation in tail coloration discussed above.

TABLE 1. Mensural data for the type series of *Dixonius hangseesom*, sp. nov. Abbreviations as in Materials and Methods, all measurements in mm.

	Holotype	Paratypes				
	CUMZ R 2003.58	CUMZ R 2003.57	CUMZ R 2003.60	CUMZ R 2003.6	ZMB 65437	IRSNB 2587
Sex	male	female	female	male	male	male
SVL	36.92	39.13	36.55	39.20	42.12	36.68
HeadL	10.63	10.63	11.08	10.99	11.29	10.24
HeadW	6.40	6.78	6.22	6.57	7.62	6.25
HeadH	4.30	4.51	4.23	4.24	4.65	4.20
OrbD	2.73	2.61	2.83	2.61	3.03	2.57
EyeEar	3.19	2.92	2.65	2.79	3.02	2.57
SnEye	3.96	4.28	4.21	4.38	4.37	4.02
NarEye	2.97	3.04	2.61	2.66	2.99	2.62
Interorb	3.65	3.93	3.23	3.25	3.89	3.26
EarL	0.89	0.89	0.84	0.77	0.84	0.91
Internar	0.95	1.27	0.90	0.92	1.21	0.89
TrunkL	15.70	16.31	14.87	17.14	18.41	17.50
ForeaL	4.88	5.07	5.19	5.62	5.54	5.11
CrusL	5.94	6.28	6.03	6.14	6.22	5.67
TailL (regen.)	49.51	33.51	33.77	46.54	51.49	47.56
TailW	3.29	3.39	3.29	3.42	4.38	3.83

Etymology

The specific epithet is derived from a transliteration of the Thai "hang" meaning tail, "see" meaning color, and "som" meaning orange. It refers to the most obvious diagnostic feature of the species, its orange tail color. The transliteration follows that of Allison (1978) as used by Cox (1991).

Observations in captivity

One paratype of *Dixonius hangseesom* (ZMB 65437), captured when about 33 mm SVL, lived ten years and two months in captivity, maintained in a daily and seasonally variable temperature regime of 16.5 C–29.8 C in the care of W. Grossmann. During this period this animal was strictly crepuscular and nocturnal in its activity cycle. It was maintained on a diverse diet of food insects including crickets, locusts, fruit flies, wax worms, and meal worms. The orange tail of this gecko became darker with age, becoming mid-brown during the last three years of its life (Figs 4–5). During the last year of its life the behavior of the specimen changed and daytime emergence from its retreat site was noted. Ultimately the gecko's movements became slowed and in the last week of life it became uncoordinated. Given the extreme longevity of this specimen—comparable to the maximum age achieved by most small-bodied gekkonids (Bowler, 1977; Rösler, 1982, 1987), we assume that this behavioral alteration and motor degradation was the result of senescence rather than a specific pathology and that *D. hangseesom* in the wild is unlikely to reach this advanced age.

Distribution and natural history

To date *Dixonius hangseesom* has only been found in Kanchanaburi Province in western Thailand. The CUMZ and IRSNB paratypes were collected on a limestone hill in bamboo forest (Fig. 7). The ZMB paratype was collected under stones after heavy rainfall near the edge of primary forest. The new species occurs sympatrically with *D. siamensis* in some localities, but it occupies more mesic microhabitats and has been found at higher elevations when the two species co-occur. *Dixonius siamensis* is typically found in drier, rocky locales, although it can occupy a fairly broad spectrum of habitats from sea level to at least 700 m (Manthey and Grossmann, 1997; Pauwels et al., 2003).

Among the other reptiles found sympatrically with the types from Sai Yok are *Cyrtodactylus peguensis*, *Gekko* cf. *siamensis*, *Gehyra fehlmanni*, *Calotes mystaceus*, *Lygo-*

FIG. 6. Adult *Dixonius hangseesom*, sp. nov. in situ on limestone at Sai Yok, Kanchanaburi Province, Thailand. Photo by Nonn Panitvong.

FIG. 7. Pathway in bamboo forest at type locality of *Dixonius hangseesom*, sp. nov. near Ban Tha Sao, Sai Yok, Kanchanaburi Province, Thailand. Photo by Montri Sumontha.

soma quadrupes, *Ramphotyphlops braminus*, *Pareas carinatus*, *Ahaetulla prasina*, *Oligodon* cf. *fasciolatus*, *Ptyas mucosa*, and *Trimeresurus kanburiensis* (M. Sumontha, G. Vogel, unpublished data; C. Schäfer, pers. comm.). Sai Yok is also the only known locality for the recently described *Cyrtodactylus tigroides*, which is apparently restricted to limestone habitats (Bauer et al., 2003). Unfortunately, existing information regarding *Dixonius hangseesom* is insufficient to determine if it is another limestone substrate endemic or if its distribution extends out of Kanchanaburi Province, either eastwards, or westwards into Myanmar. However, Kanchanaburi and adjacent areas do harbor a number of endemic species including Kitti's hog-nosed bat, *Craseonycterus thonglongyai* Hill, 1974 (Humphrey and Bain, 1990) and the pitviper *Trimeresurus kanburiensis* Smith, 1943 (Cox, 1991). The discovery of two new geckos from the province, serves to further highlight the importance of Kanchanaburi as a center of diversity and endemism within Thailand.

Dixonius hangseesom is threatened by the pet trade and is available, along with *D. siamensis*, from animal sellers at Jatujak Market in Bangkok. It is hoped that its identification as a distinct species will permit its eventual protection.

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APPENDIX

Comparative Material Examined

All material from Thailand.

Dixonius melanostictus

FMNH 178232; Sara Buri Province.

Dixonius siamensis

Gernot Vogel (GV), 2 uncatalogued specimens; Kanchanaburi Province, Nongbuwa Village near Kanchanaburi. GV, uncatalogued specimens, Tak Province, near border with Myanmar, ca. 600 m. IRSNB 15155; Phetchaburi Province, Kaeng Krachan District, Ban Khao Kling. IRSNB 16642; Chiang Mai Province, Muang District, Chiang Mai City. IRSNB 16643; Chiang Mai Province, Doi Saket District, Doi Saket. IRSNB 16645; Phetchaburi Province, Ban Lat District, Ban Nong Ipho, foot of Khao Loun. CUMZ R 2003.59; Kanchanaburi Province, Sai Yok District, near Sai Yok Noi Waterfall. FMNH 177730, 177732, 177735, 177766, 177796; Chon Buri Province; CAS 95254–57; Tak Province, Yan Hee Dam.

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Parasitic Nematodes Recorded from Amphibians and Reptiles in Japan

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Abstract: A list of nematode species parasitic in amphibians and reptiles of Japan is presented. A total of 82 species are recorded, of which 51 are from amphibians, 42 from reptiles, and 11 from both groups. Three avian and six mammalian nematode species are known to utilize amphibians and reptiles as paratenic hosts.

Key words: Nematoda; Parasite; Amphibia; Reptilia; Japan; Species list

As a part of the species census in Japan under the auspices of the Union of Japanese Societies for Systematic Biology, we were assigned to count the number of parasitic nematode species hitherto known from Japan. The nematode species parasitic in freshwater fishes were already reviewed by Shimazu (1998), and those parasitic in birds and mammals were reviewed by Asakawa and Hasegawa (2003). Here we present a list of nematodes hitherto recorded from the Japanese amphibians and reptiles.

Early research on the nematode parasites of the Japanese herpetofauna was carried out by K. Morishita, S. Yamaguti, J. K. Wilkie, and A. Pearse during the first half of the 20th century. However, very little attention was paid to taxonomic diversity and nomenclature in this group of organisms by subsequent researchers in Japan. Interest in nematodes parasitic in amphibians and reptiles was not strong among Japanese researchers. As a result, names of taxa invalidated outside Japan have been occasionally used in Japanese literature without critical reconsiderations (cf. Ishii, 1973; Kagei, 1973). Moreover, absence of type material has often caused difficulties in revising the taxonomy of problematic groups (cf. Schad, 1962; Sprent, 1978).

Checklists have been presented for helminth parasites of amphibians and reptiles of Japan several times (Kagei, 1973; Kamegai and Ichihara, 1973; Uchida, 1975, 1976). With respect to the nematodes parasitic in these vertebrates a comprehensive synopsis was also published by Baker (1987) for taxa of the world recognized then. However, these checklists are now outdated due to the results of subsequent research. Because of these taxonomic and nomenclatural problems, study of the nematode fauna in the Japanese amphibians and reptiles lags behind that in other vertebrate groups in Japan.

In listing nematode species here, we largely follow the molecular-based classification of De Ley and Blaxter (2002). However, this system is still incomplete in some details, and conflicts with traditional classification in the treatment of a number of taxa including several genera found in Japanese amphibians and reptiles. We have classified these genera provisionally.

Nematodes considered here are principally those having an adult stage in the amphibians and reptiles captured in Japan. Nematode species whose larval forms were recorded from these vertebrates are also listed, even though their adults are confined to other groups of hosts, such as mammals and birds. In recent years, various exotic amphibians and reptiles have been imported, often as pets, and some of them have established feral populations in the field (Ota, 1999; Lever, 2003). Nematodes reported from these introduced species are also considered in this study. In contrast, those found in exotic amphibians and reptiles

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in captivity or temporarily escaped from captivity are excluded (cf. Hasegawa and Katsuren, 1998).

Table 1 shows the nematode species hitherto recorded from the herpetofauna of Japan. The exact number of nematode species cannot be determined because identification of members of some genera is not reliable at the species level. Nevertheless, at least 82 nematode species are parasitic in the Japanese herpetofauna, of which 51 parasitize amphibians, 42 reptiles, and 11 both groups. Besides these, the larvae of three nematode species, which are parasitic in birds and six which are parasitic in mammals in the adult stage are known to utilize amphibians and reptiles as paratenic hosts. One species from amphibians and three from reptiles are recorded as pseudoparasites.

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TABLE 1. A list of parasitic nematodes hitherto recorded from amphibians and reptiles of Japan (scientific name of parasite followed by host name and site in host in parenthesis, and reference in brackets).

Phylum NEMATODA

Class ENOPLEA

Subclass Dorylaimia

Order Dioctophymatida

Family Dioctophymatidae

Dioctophyme renale (Goez, 1782)* [larva] (*Andrias japonicus*; stomach wall) [Hasegawa et al., 2002]

Order Trichinellida

Family Capillariidae

Amphibiocapillaria bufonis (Morishita, 1926) (*Bufo japonicus*; small intestine) [Goldberg and Bursey, 2002]

Amphibiocapillaria tritonispunctati Diesing, 1851 (*Hynobius nebulosus*, *H. stejnegeri*, *Onychodactylus japonicus*, *Andrias japonicus*, *Cynops pyrrhogaster*, *C. ensicauda*; small intestine) [Baker, 1987; Hasegawa, 1989a, 1990; Hasegawa et al., 2002]

Aonchotheca buccalis Yamaguti, 1943 (*Bufo japonicus*; buccal cavity) [Uchida, 1975; Baker, 1987]

Aonchotheca sp. (*Rana narina*, *R. amamiensis*; buccal cavity) [Hasegawa, 1989a, 1990; Hasegawa and Iwatsuki, 1993a]

Paracapillaria kuntzi Moravec et Gibson, 1986 (*Elaphe quadrivirgata*, *Rhabdophis tigrinus tigrinus*; small intestine) [Goldberg et al., 2004]

Paracapillaria sp. (*Rana narina*; small intestine) [Hasegawa, 1989a; Hasegawa and Iwatsuki, 1993a]

Capillariidae gen. sp. (*Trimeresurus flavoviridis*, *T. okinavensis*; small intestine) [Hasegawa, 1985]

Capillariidae gen. sp. (*Elaphe quadrivirgata*; small intestine) [Kagei, 1973]

Class CHROMADOREA

Subclass Chromadoria

Order Rhabditida

Suborder Spirurina

Superfamily Dracunculoidea

Family Dracunculidae

Kamegainema cingula (Linstow, 1902) (*Andrias japonicus*, skin) [Hasegawa et al., 2000]

Superfamily Gnathostomatoidea

Family Gnathostomatidae

Gnathostoma doloresi Tubangui, 1925* [larva] (*Hynobius naevius*, *H. stejnegeri*, *Cynops ensicauda*, *Rana narina*, *R. subaspera*, *Amphiesma pryeri pryeri*, *Dinodon semicarinatum*, *Trimeresurus elegans*, *T. okinavensis*, *T. flavoviridis*; viscera, muscle) [Kagei, 1973; Uchida, 1976; Hasegawa, 1985, 1990; Hasegawa and Iwatsuki, 1993a]

Gnathostoma nipponicum Yamaguti, 1941* [larva] (*Rhabdophis tigrinus tigrinus*; muscle) [Koga and Ishii, 1981]

Gnathostoma spinigerum Owen, 1836* [larva] (*Rana catesbeiana*, *R. nigromaculata*) [Uchida, 1975]

Spiroxys hanzaki Hasegawa et al., 1998 (*Andrias japonicus*; stomach) [Hasegawa et al., 1998, 2002]

Spiroxys japonica Morishita, 1926 (*Rana japonica*, *R. nigromaculata*, *R. rugosa*; duodenum, stomach) [Goldberg and Bursey, 2002]

Superfamily Oxyuroidea

Family Pharyngodonidae

Parapharyngodon japonicus Bursey et Goldberg, 1999 (*Onychodactylus japonicus*; rectum) [Bursey and Goldberg, 1999]

Parapharyngodon sp. (*Ateuchosaurus pellopleurus*, *Eumeces marginatus marginatus*; rectum) [Hasegawa, 1985, 1988]

Pharyngodon polypedatis Yamaguti, 1941 (*Buergeria buergeri*; rectum) [Goldberg and Bursey, 2002]

Skrjabinodon sp. or spp. (*Gekko hokouensis*, *Gehyra mutilata*, *Hemidactylus frenatus*, *H. bowringii*, *Polypedates leucomystax*; rectum) [Hasegawa, 1985, 1990]

Pharyngodonidae gen. sp. (*Rhacophorus viridis viridis*; rectum) [Hasegawa, 1989a]

Pharyngodonidae gen. sp. (*Rhacophorus viridis amamiensis*; rectum) [Hasegawa, 1989a (referred to as *Skrjabinodon* sp.)]

Superfamily Camallanoidea

Family Camallanidae

Camallanus sp. (*Rana limnocharis*; rectum) [Hasegawa, 1986]

Serpinema intermedius (Hsü et Hoeppli, 1931) (*Chinemys reevesii*; intestine) [Baker, 1987]

Serpinema microcephalus (Dujardin, 1845) (*Trachemys scripta*; stomach) [Ishida et al., 2004]

Superfamily Physalopteroidea

Family Physalopteridae

Abbreviata sp. (*Dinodon rufozonatum walli*, *Trimeresurus elegans*; stomach) [Hasegawa, 1985]

Pseudabbreviata yambarensis Hasegawa et Otsuru, 1984 (*Japalura polygonata polygonata*; duodenum) [Hasegawa, 1985]

Physalopteridae gen. sp. (*Andrias japonicus*†, intestine) [Hasegawa et al., 2002]

Superfamily Spiruroidea

Family Spirocercidae

Ascarops strongylina (Rudolphi, 1819)* [larva] (*Japalura polygonata polygonata*; stomach wall) [Hasegawa, 1992b]

Superfamily Habronematoidea

Family Hedruridae

Hedruris ijimai Morishita, 1926 (*Rana japonica*, *R. nigromaculata*, *R. rugosa*; duodenum) [Uchida, 1975; Baker, 1987]

Hedruris miyakoensis Hasegawa, 1989 (*Scincella boettgeri*, *Bufo gargarizans miyakonis*; stomach) [Hasegawa, 1989b; Hasegawa and Iwatsuki, 1993b]

Family Cystidicolidae

Spinitectus ranae Morishita, 1926 (*Rana nigromaculata*, *R. rugosa*; stomach) [Uchida, 1975; Baker, 1987]

Superfamily Acuarioidea

Family Acuariidae

Dispharynx nasuta (Rudolphi, 1819)§ [larva] (*Buergeria japonica*, *Microhyla ornata*, *Rana limnocharis*, *Gekko hokouensis*, *Hemidactylus frenatus*, *Japalura polygonata polygonata*, etc.; alimentary canal wall, abdominal muscle, etc.) [Hasegawa, 1984, 1985]

Dispharynx sp.§ [larva] (*Buergeria japonica*, *Microhyla ornata*, *Rana limnocharis*, *Gekko hokouensis*, *Hemidactylus frenatus*, *Japalura polygonata polygonata*, etc.; alimentary canal wall, abdominal muscle, etc.) [Hasegawa, 1984, 1985, 1992a]

Acuariidae gen. sp.§ [larva] (*Tachydromus smaragdinus*; abdominal muscle) [Hasegawa, 1985]

Superfamily Filarioidea

Family Onchocercidae

Icosiella kobayashii Yamaguti, 1941 (*Rana limnocharis*; subcutaneous tissue, connective tissue) [Uchida, 1975; Baker, 1987]

Icosiella sasai Hayashi, 1960 (*Rana subaspera*; connective tissue) [Hasegawa, 1990]

Superfamily Ascaridoidea

Family Ascarididae

Hexametra quadricornis (Wedl, 1961) (*Dinodon semicarinatum*, *Trimeresurus flavoviridis*, *T. okinavensis* etc.; small intestine, stomach. *Gekko hokouensis*, *Tachydromus tachydromoides*; subcutaneous tissue, body cavity. *Rana japonica*, *R. ornativentris*, *R. subaspera*; body cavity) [Hasegawa, 1985, 1990; Telford, 1997; Goldberg et al., 2004]

Family Raphidascarididae

Paraheterotyphlum cf. *ophiophagus* Schmidt et Kuntz, 1973 (*Laticauda semifasciata*, esophagus) [Kagei, 1973]

Superfamily Cosmocercoidea

Family Cosmocercidae

Aplectana cf. *macintoshi* Stewart, 1914 (*Rana ishikawae*; rectum) [Hasegawa, 1990]

Cosmocerca japonica Yamaguti, 1938 (*Rana japonica*, *R. nigromaculata*, *R. narina*, *R. ornativentris*, *R. rugosa*, *R. tagoi*, *Bufo japonicus*, *Hyla japonica*, *Microhyla ornata*, *Buergeria japonica*, *Polypedates leucomystax*; *Rhacophorus schlegelii*, *Cynops ensicauda*; rectum) [Hasegawa, 1984, 1989a; Hasegawa and Iwatsuki, 1993a; Goldberg et al., 1994, 1996; Goldberg et al., 1997; Goldberg and Bursey, 2002]

Cosmocercella iwatsukii Hasegawa, 1989 (*Rhacophorus viridis viridis*; rectum, small intestine) [Hasegawa, 1989a]

Cosmocercoides pulcher Wilkie, 1930 (*Bufo japonicus*, *B. gargarizans miyakonis*, *Rana holsti*, *R. ishikawae*, *R. japonica*, *R. ornativentris*; rectum. *Elaphe quadrivirgata*†, *Rhabdophis tigtinus*†; small intestine) [Hasegawa, 1984, 1989a; Hasegawa and Iwatsuki, 1993b; Goldberg and Bursey, 2002, 2003; Goldberg et al., 2004]

Cosmocercoides tridens Wilkie, 1930 (*Echinotriton andersoni*, *Cynops ensicauda*; rectum) [Hasegawa, 1989a, 1989c, 1990]

Gyrinicola japonica Yamaguti, 1938 (*Rana rugosa* [tadpole]; intestine) [Uchida, 1975; Baker, 1987]

Raillietnema rhacophori Yuen, 1965 (*Polypedates leucomystax*; rectum) [Hasegawa, 1993]

Family Kathlaniidae

Falcaustra japonensis Yamaguti, 1935 (*Mauremys japonica*, *Chinemys reevesii*,? *Mauremys mutica*; rectum) [Baker, 1987; Hasegawa, unpublished observation]

Falcaustra sp. (*Rana subaspera*; small intestine) [Hasegawa, 1990]

Megalobatrachonema nipponicum Yamaguti, 1941 (*Andrias japonicus*; small intestine) [Hasegawa et al., 2002]

Pseudoxyascaris japonicus Uchida et Itagaki, 1979 (*Onychodactylus japonicus*; small intestine) [Uchida and Itagaki, 1979]

Superfamily Heterakoidea

Family Heterakidae

Meteterakis amamiensis Hasegawa, 1990 (*Rana ishikawae*, *Ateuchosaurus pellopleurus*, *Eumeces marginatus oshimensis*; rectum) [Hasegawa, 1990]

Meteterakis ishikawanae Hasegawa, 1987 (*Rana ishikawae*, *Geoemyda japonica*, *Ateuchosaurus pellopleurus*, *Japalura polygonata polygonata*; rectum) [Hasegawa, 1989a; 1992a; Nakachi and Hasegawa, 1992]

Meteterakis japonica (Wilkie, 1930) (*Bufo japonicus*, *B. gargarizans miyakonis*, *Rana japonica*, *R. ornativentris*, *Eumeces latiscutatus*, *Tachydromus tachydromoides*; rectum) [Hasegawa, 1984, 1989a; Hasegawa and Iwatsuki, 1993b; Telford, 1997; Goldberg and Bursey, 2002]

Meteterakis sp. (?*Eumeces kishinouyei*; ?rectum) [Hasegawa, 1992b]

Family Ascarididae

Strongyluris calotis Baylis et Daubney, 1923 (*Japalura polygonata polygonata*, *J. polygonata ishigakiensis*; lung, rectum) [Hasegawa, 1985 (referred to as *Strongyluris japalurae* (Yamaguti, 1935); 1992a]

Superfamily Seuratoidea

Family Seuratidae

Skrjabinelazia machidai Hasegawa, 1984 (*Gekko hokouensis*; intestine, stomach) [Hasegawa, 1985]

Family Quimperiidae

Wakubitinema toyamai Hasegawa, 1988 (*Rana namiyei*; small intestine) [Hasegawa, 1989a]

Suborder Tylenchina

Superfamily Strongyloidoidea

Family Strongyloididae

Strongyloides mirzai Singh, 1954 (*Trimeresurus flavoviridis*; small intestine) [Hasegawa, 1985]

Strongyloides sp. or spp. (*Chirixalus eiffingeri*, *Rana nigromaculata*, *Japalura polygonata ishigakiensis*, *Ateuchosaurus pellopleurus*; rectum) [Hasegawa, 1985, 1989a, 1992a, unpublished observation]

Family Rhabdiasidae

Angiostoma onychodactyla Bursey et Goldberg, 2000 (*Onychodactylus japonicus*; rectum) [Bursey and Goldberg, 2000]

Entomelas cf. *markovi* Sczerbak et Sharpilo, 1969 (*Tachydromus tachydromoides*, *Eumeces latiscutatus*; lung) [Telford, 1997 (referred to as *Rhabdias* sp.); Hasegawa, unpublished observation]

Neoentomelas asatoi Hasegawa, 1989 (*Ateuchosaurus pellopleurus*; lung) [Hasegawa, 1989b, 1990, 1992a]

Rhabdias agkistrodonis Sharpilo, 1976 (*Trimeresurus flavoviridis*, *T. okinavensis*, ?*T. elegans*; lung) [Kuzmin, 1999]

Rhabdias horigutii Yamaguti, 1943 (*Rhabdophis tigrinus tigrinus*, *Gloydius blomhoffii*; lung) [Kagei, 1973; Baker, 1987; Goldberg et al., 2004]

Rhabdias incerta Wilkie, 1930 (*Bufo japonicus*, *B. gargarizans miyakonis*; lung) [Hasegawa, 1984; Hasegawa and Iwatsuki, 1993b; Goldberg and Bursey, 2002]

Rhabdias nipponica Yamaguti, 1935 (*Hyla japonica*, *Rana japonica*, *R. limnocharis*, *R. narina*, *R. nigromaculata*, *R. ornativentris*, ?*R. pirica*, *R. tagoi*, *Buergeria buergeri*, ?*B. japonica*; lung) [Hasegawa and Iwatsuki, 1993a; Goldberg et al., 1997; Goldberg and Bursey, 2002, 2003; Asakawa, 2002]

Rhabdias rhacophori Yamaguti, 1940 (*Rhacophorus arborea*, *R. schlegelii*; lung) [Uchida, 1975; Baker, 1987]

Rhabdias tokyoensis Wilkie, 1930 (*Cynops pyrrhogaster*, *C. ensicauda*; lung) [Hasegawa, 1984, 1990]

Rhabdias sp. (*Japalura polygonata ishigakiensis*; lung) [Hasegawa, 1985]

Suborder Rhabditina

Superfamily Strongyloidea

Family Ancylostomatidae

Kalicephalus brachycephalus Maplestone, 1931 (*Amphiesma pryeri pryeri*, *Dinodon rufozonatum walli*, *Rhabdophis tigrinus tigrinus*, *Gloydius blomhoffii*; rectum) [Hasegawa, 1985, 1992a; Goldberg et al., 2004]

Kalicephalus costatus indicus Ortlepp, 1923 (*Amphiesma vibakari vibakari*, *Cyclophiops semicarinatus*, *Rhabdophis tigrinus tigrinus*; small intestine) [Hasegawa, 1985; Goldberg et al., 2004]

Kalicephalus laticaudae Yamaguti, 1935 (*Laticauda laticaudata*, *L. semifasciata*; small intestine) [Kagei, 1973]

Kalicephalus posterovulvus Schad, 1962 (*Amphiesma pryeri pryeri*, *Dinodon semicarinatum*; esophagus) [Hasegawa, 1985, 1990]

Kalicephalus sinensis Hsü, 1934 (*Elaphe climacophora*, *E. conspicillata*, *E. quadrivirgata*; esophagus, small intestine) [Goldberg et al., 2004]

Kalicephalus viperae chungkingensis Hsü, 1934 (*Trimeresurus okinavensis*; esophagus) [Hasegawa, 1985, 1990]

Kalicephalus sp. (*Dinodon rufozonatum walli*; esophagus) [Hasegawa, 1985]

Family Trichostrongylidae

Batrachonema synaptospicula Yuen, 1965 (*Rana narina*, *Rhacophorus viridis viridis*; small intestine) [Hasegawa, 1989a; Hasegawa and Iwatsuki, 1993a]

Oswaldocruzia insulae Morishita, 1926 (*Bufo japonicus*, *B. gargarizans miyakonis*, *Hyla japonica*, *Rana rugosa*; small intestine) [Hasegawa, 1989a; Hasegawa and Iwatsuki, 1993b; Goldberg et al., 1994; Goldberg and Bursey, 2002]

Oswaldocruzia hoepplii Hsü, 1935 (*Rana limnocharis*; small intestine) [Hasegawa, 1989a]

Oswaldocruzia cf. *japalurae* Jing et Lin, 1980 (*Japalura polygonata ishigakiense*; small intestine) [Hasegawa, 1985]

Oswaldocruzia socialis Morishita, 1926 (*Rana japonica*, *R. nigromaculata*, *R. ornativentris*, *Rhacophorus arboreus*, *Rh. schlegelii*, *Bufo japonicus*, *Tachydromus tachydromoides*, *Elaphe quadrivirgata*†, *Rhabdophis tigrinus*†; small intestine, rectum) [Telford, 1997 (referred to as *Oswaldocruzia filiformis*); Goldberg et al., 1997; Goldberg and Bursey, 2002 2003; Goldberg et al., 2004]

Oswaldocruzia yezoensis Morishita, 1926 (*Rana pirica*, small intestine) [Asakawa, 2002]

Family Metastrongylidae

Angiostrongylus cantonensis (Chen, 1935* [larva] (*Bufo gargarizans miyakonis*, *Rana limnocharis*, *R. catesbeiana*, *Polypedates leucomystax*; liver) [Uchida, 1976; Otsuru, 1977]

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* Adult stage parasitic in mammals.

§ Adult stage parasitic in birds.

† Pseudoparasitism.

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Notes on Reproduction and Variation in the Blue-tailed Lizard, *Eumeces elegans* (Reptilia: Scincidae), on Kita-kojima Island of the Senkaku Group, Ryukyu Archipelago

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Abstract: A field survey was carried out for a population of *Eumeces elegans* on Kita-kojima Island of the Senkaku Group, Ryukyu Archipelago. Data for several reproductive parameters, such as clutch size, egg size, hatchling size, and hatching date, are provided for the first time for the Japanese populations of this species. Variations in midbody scale rows and hatchling coloration between the Senkaku populations and the Taiwanese and continental populations are clarified and their geohistorical significance is discussed briefly.

Key words: *Eumeces elegans*; Scincidae; Reproduction; Midbody scale row; Hatchling coloration; Senkaku Group; Japan

The Senkaku Group is an assemblage of eight uninhabited islets, and is located ca. 150 km NNW of the Yaeyama Group and ca. 160 km ENE of Taiwan. Since the end of the 19th Century, several surveys have been made of the fauna and flora of the Senkaku Group, yielding a series of natural history specimens for the study of their taxonomic diversity (see Ota et al. [1993] and Yokohata and Yokota [2000] for review). Even so, however, available specimens are still far from being sufficient for reliable estimation of the degree of divergence in each lineage between this island group and adjacent regions (Ota et al., 1993; Shy and Ng, 1998; Motokawa et al., 2001). Likewise, very little is known of the ecology of organisms on the Senkaku islands.

A brief visit to the Senkaku Group was organized by a commercial tourist company, and this gave me an opportunity to carry out a herpetological survey on Kita-kojima Island, a small islet of the group (0.3 km^2 in area and 124.5 m in maximum elevation) consisting chiefly of a sandstone bed with an upheaved coral reef zone along the northern half of the coast (Kizaki, 1985). In this paper I report some aspects of reproductive habits and variations of the skink, *Eumeces elegans*, observed at this opportunity. This species is broadly distributed in Taiwan and continental China (Zhao and Adler, 1993; Zhao et al., 1999). Within Japanese territory, however, its range is confined to the Senkaku Group (Hikida, 1989; Ota et al., 1993).

I visited Kita-kojima Island from 07:30 to 17:00 on 16 June 1995. During this period, I surveyed as many types of habitats as possible, examining both the ground surface and under rocks for the skink. Each individual found was collected whenever possible. When a nest was located, the posture of a brooding individual (sensu Somma, 1988) in relation to eggs was recorded. Immediately after collection, measurements of lizards and eggs were taken to nearest 0.01 mm and 0.01 g with dial calipers and an electronic balance. The number of transverse scale rows was also examined at midbody for each lizard. Maturity status of each specimen was determined by gonadal investigation following Okada et al. (1992). All voucher specimens were deposited in the Zoological Collection of the Kyoto University Museum (KUZ). A total of 31 hatchlings of *E. elegans* from Taiwan, including eight reported by Kato and Ota (1994), were used for comparisons.

Clutches found were brought back to the laboratory on Okinawajima Island, where they were incubated separately at room temperature and were monitored twice per day until hatching. On hatching, each hatchling was

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measured in the same way as above and was photographed for the purpose of recording the coloration.

Two adult males (59.2 and 80.9 mm in snout-vent length [SVL], 4.84 and 11.47 g in mass, and 27 and 28 in body scale rows [BSR]), four adult females (57.5–73.1 mm in SVL, 3.60–6.20 g in mass, and 28 [n=3] or 29 [n=1] in BSR), and one juvenile (31.5 mm in SVL, 0.63 g in mass, and 28 in BSR) were collected while they were active on the ground. Four nests, each a hollow containing one adult female and six or seven eggs, were also located beneath coral rocks. In each nest, the female was in a half-coiled posture, surrounding the clutch with its whole body and tail like brooding females of other conspecific (Wang, 1966; Kato and Ota, 1994) and congeneric populations (Somma, 1990; Hosono and Hikida, 1999). All females but one, which escaped from a subsequent attempt at capture, and all clutches were collected and measured (Table 1). In all three females collected, the BSR number was 28.

The clutches incubated in the laboratory hatched from 22 to 27 June (Table 1). The hatchlings, as well as the one juvenile collected during the field survey (see above), exhibited five longitudinal light lines against a dark gray ground color on the body. The distal half of the tail was light blue, whereas the proximal half was dark gray or tan. Posterior extensions of dorso-median and dorso-lateral light lines from the body were evident in the proximal 1/4–1/3 of tail, but then faded to become invisible posteriorly (Fig. 1A). In the hatchlings from Taiwan, the body ground color was almost black, and the whole tail, as well as the posterior and distal portions of the hind limbs, was dark blue or violet. Posterior extensions of light lines from the body were visible in no more than the proximal 1/5 of the tail (Fig. 1B).

The one juvenile collected in the survey was similar in SVL to hatchlings obtained in the

FIG. 1. Hatchlings of *Eumeces elegans* from Kita-kojima Island (A) and Taiwan (B).

TABLE 1. Measurements (in mm and g) of brooding females, eggs, and hatchlings of *Eumeces elegans* collected on 16 June 1995 from Kita-kojima Island of the Senkaku Group, Japan. Values are given as x̄±SD, followed by ranges in parentheses.

Nest no.	Females*			Clutch size	Eggs*			Hatching date	Hatchlings**		
	SVL	Tail***	Mass		Length	Width	Mass		SVL	Tail	Mass
1	70.0	46.0+40.5	6.51	6	16.76±0.55	11.87±0.05	1.28±0.07	June	28.70±0.62	44.56±0.71	0.51±0.02
					(16.2–17.6)	(11.8–11.9)	(1.18–1.38)	22–24	(27.9–29.4)	(43.6–45.3)	(0.49–0.53)
2	74.3	51.0+46.5	7.04	7	15.63±0.52	11.90±0.16	1.22±0.06	June	30.24±0.38	46.38±1.14	0.61±0.00
					(15.0–16.6)	(11.7–12.1)	(1.15–1.30)	23	(29.8–30.8)	(44.5–47.3)	(0.61–0.62)
3	73.5	22.6+43.7	5.59	6	16.20±0.70	11.72±0.47	1.16±0.12	June	28.70±0.29	43.83±1.12	0.49±0.03
					(15.0–16.9)	(10.9–12.2)	(0.94–1.28)	26–27	(28.4–29.0)	(42.3–45.0)	(0.46–0.52)
4	—	—	—	6	17.10±0.54	11.92±0.20	1.32±0.06	June	29.53±0.31	44.65±1.31	0.50±0.02
					(16.4–17.9)	(11.8–12.3)	(1.27–1.43)	24	(29.1–29.8)	(43.0–46.2)	(0.48–0.52)

* Measured immediately after being found in nests.

** Measured immediately after hatching.

*** Intact portion+regenerated portion.

laboratory (31.5 mm vs. 27.9–30.8 mm), and it is obvious that this juvenile had hatched slightly before the survey. This and the results of captive observations indicate that in the Kita-kojima population of *E. elegans* hatching occurs at least from middle to late June. This period, while overlapping the hatching season reported for the continental *E. elegans* populations (Zhao et al., 1999), seems to be substantially earlier than that in most conspecific populations in Taiwan (Lin and Lue, 1989; Lue and Chen, 1989; Ota, unpublished data), although eggs from one female from northern Taiwan hatched on 14 June in captivity (Kato and Ota, 1994). Likewise, clutch size in the Kita-kojima populations (6–7), overlapping the range in the continental populations (4–11: Zhao et al., 1999), may be smaller than that in the Taiwanese populations (8–9: Lue and Chen, 1989; Kato and Ota, 1994; Ota, unpublished data).

Two of the four adult females found active on the ground had distinctly extended oviducts, whereas in the remaining two females, oviducts were hypertrophied but not much extended. Values of condition index (defined as [BW/SVL^3]×105) in the former (1.46 and 1.61) were distinctly smaller that those in the latter (2.07 and 2.22), and were within the range of corresponding values in the three nesting females (1.41–1.90). These may suggest that only the former two females bred in 1995, and that the reproductive frequency in mature females of the Kita-kojima population of *E. elegans* is lower than annual as in some insular populations of *E. okadae* (see Hasegawa [1984, 1994]).

The reptile fauna of the Senkaku Group is more similar to that of Taiwan and continental China rather than to that of the other parts of the Ryukyu Archipelago, as a result of the presence of a few taxa exclusively shared between this island group and those two regions (i.e., *Eumeces elegans*, *Dinodon rufozonatum rufozonatum*, and *Elaphe carinata carinata*: Ota et al., 1993; Ota, 2000). Such a geographic pattern of faunal similarity is usually explained by assuming dry-land connections between the Senkaku Group, Taiwan, and the continent during the Late Pleistocene Glaciation (LPG: 0.015–0.02 Mybp): in this period, the sea level is generally considered to have been lowered by ca. 120 m (e.g., Fairbanks, 1989), a value slightly greater than the maximum depth of the strait between the three regions (ca.110–115 m: Maritime Safety Agency, Japan, 1978). However, Ohshima (1990), on the basis of geomorphological and sedimentological data, estimated the extent of the sea level lowering during the LPG to be no more than 80 m, and Motokawa and Hikida (2003) recently argued that the geographic genetic variations among the *Eumeces japonicus* populations of Kyushu and adjacent islands favor this estimation. If this is the case, the Senkaku Group whould have remained isolated from Taiwan or the continent even during the LPG.

Ota et al. (1993) pointed out a slight difference in midbody scale rows between the Senkaku (mostly 28) and the Taiwanese populations of *E. elegans* (mostly 26). This difference was verified by the additional specimens obtained in the present survey (see above). Moreover, the majority of the continental specimens (47/66) has proved to possess 26 scale rows around the midbody (Zhao et al., 1999). These differences and the differences in hatchling coloration between the Senkaku and the Taiwanese populations also seem to favor Ohshima's (1990) estimation. However, because both body scale row number and the body coloration may rapidly diverge in scincid lizards (e.g., Ota et al., 1994; Bruna et al., 1996; Motokawa et al., 2001), further comparative studies on the Senkaku, Taiwanese, and continental populations of *E. elegans*, particularly using biochemical and molecular techniques, are desired to establish a more convincing hypothesis on the geohistorical relationships of these three regions.

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A Bioassay of the Attractive Effect of Mouse Urine on the Habu, *Trimeresurus flavoviridis*

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Abstract: A bioassay of the attractive effect of mouse urine on the habu, *Trimeresurus flavoviridis*, was carried out by trapping in the field and by nighttime observations of foraging behavior in an outdoor pen. No habus and nine *Dinodon semicarinatum*, a colubrid snake, were captured by traps baited with mouse urine during a period of about six months, whereas 12 habus and nine *D. semicarinatum* were captured by control traps baited with a live mouse. Nighttime observations of foraging behavior of habus suggested that mouse urine attracts a habu, but is not attractive enough to induce it to enter a trap.

Key words: Habu; *Trimeresurus flavoviridis*; Attractant; Mouse urine; Trapping; Foraging behavior

In order to eradicate the habu, *Trimeresurus flavoviridis,* by trapping more effectively and conveniently (Shiroma, 1989; Shiroma and Akamine, 1999), attractants for habu, in place of live mice and rats, have been studied during the past two decades (Hayashi et al., 1979; Hattori et al., 1999; Kihara et al., 1979; Niwa et al., 1999; Shiroma, 1994, 1998). Shiroma (1994) showed that it is possible to assay attractive effects on habus using a Y-maze apparatus in the laboratory and that mouse feces attract a habu. Shiroma (1998) also demonstrated that the attractive effects of mouse feces are derived mainly from the urine.

In the present study, a bioassay of the attractive effects of mouse urine on habus was carried out by trapping in the field and by nighttime observations of foraging behavior of habus in an outdoor pen.

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METHODS

Mouse urine was collected from cages in which approximately 100 mice (ICR strain) were kept. A fine aluminum screen (1×1 mm mesh) was spread on the floor of the cage (Shiroma 1998). The screen separated mouse urine and excrement, and only the urine was collected in a bottle (about 20 ml/day). The collected mouse urine was bottled and kept at 5C in a refrigerator for 1–13 days until use. In the field, mouse urine set in a trap was evaporated from a bottle through a towel wick (Fig. 1). In the outdoor pen experiments, mouse urine collected on the previous day was used in the same manner as in the field experiment.

In the field trapping, Okinawa-style four-side entrance habu traps (FSE trap) were used (Fig. 2). This trap is 50×50×15 cm in size, and

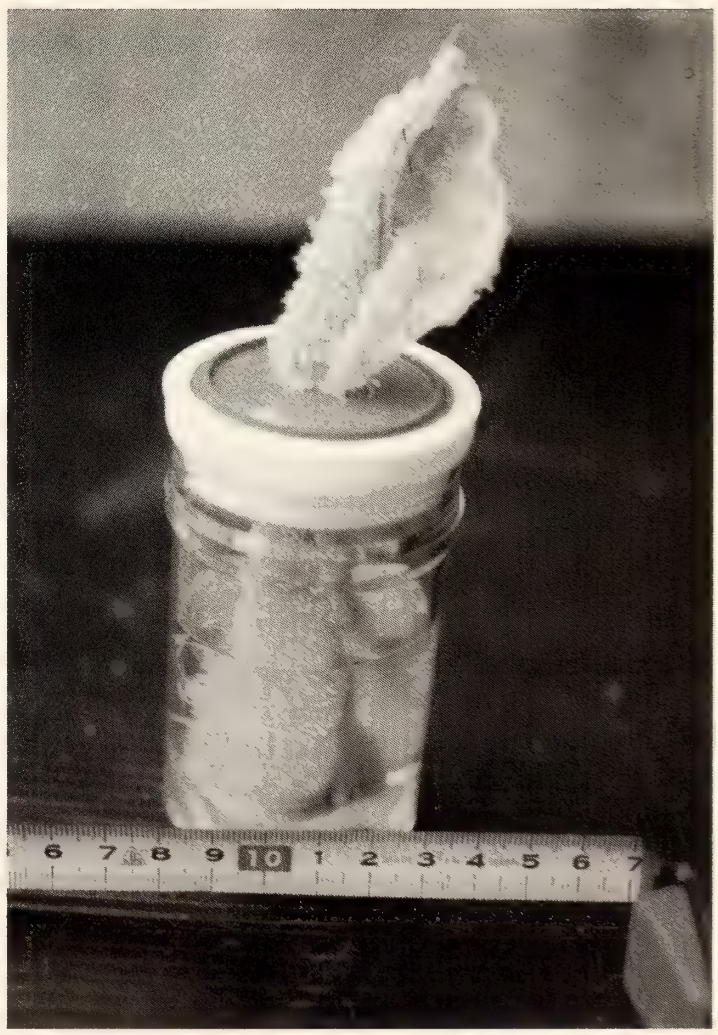

FIG. 1. Mouse urine contained in a plastic bottle. The urine evaporates through a towel wick.

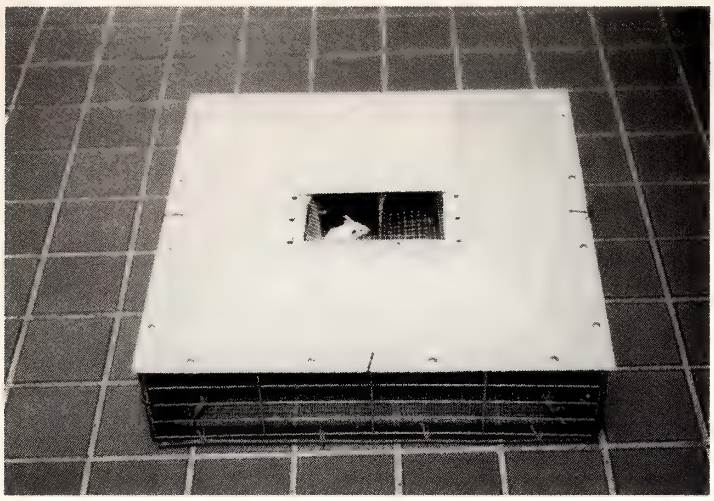

FIG. 2. Okinawa-style four-side entrance habu trap (FSE trap; 50×50×15 cm). A mouse cage is placed at the center of the trap.

the four sides of the trap are triangular prism-shaped entrances with a 1×1 mm mesh aluminum screen attached as a flap. A mouse is kept in a cage (15×15×10 cm) placed at the center of the trap. The mouse in the trap can be seen from outside the trap. Ten FSE traps baited with a live mouse and ten traps baited with mouse urine were located alternately at more than 20 m intervals along a mountain path in the forest of Ginama, Kunigami Village, in the northern part of Okinawa Island (Fig. 3). Trapping was started on 24 March 1994 and continued until 11 August 1994. The traps were checked for captives every two weeks, when the mouse was tended to and mouse urine was replaced with fresh urine.

Nighttime observations of foraging behavior of habus were conducted in an outdoor pen (about 306 m^2), where 22 shelters (50×100×100 cm) were provided and approximately 30 habus were kept (Fig. 4). The outdoor pen was located at Ozato, Ozato Village, Okinawa, Japan (this facility belongs to the habu study section, Okinawa Prefectural Institute of Health and Environment). In the outdoor pen, eight FSE traps baited with mice had usually been placed to catch habus for laboratory experiments. The traps were checked every morning except Sundays. Captured habus were used for bioassay experiments of attractants for about two weeks, and then returned to the outdoor pen after feeding on one to three mice. On 11 August 1994, two FSE habu traps baited with

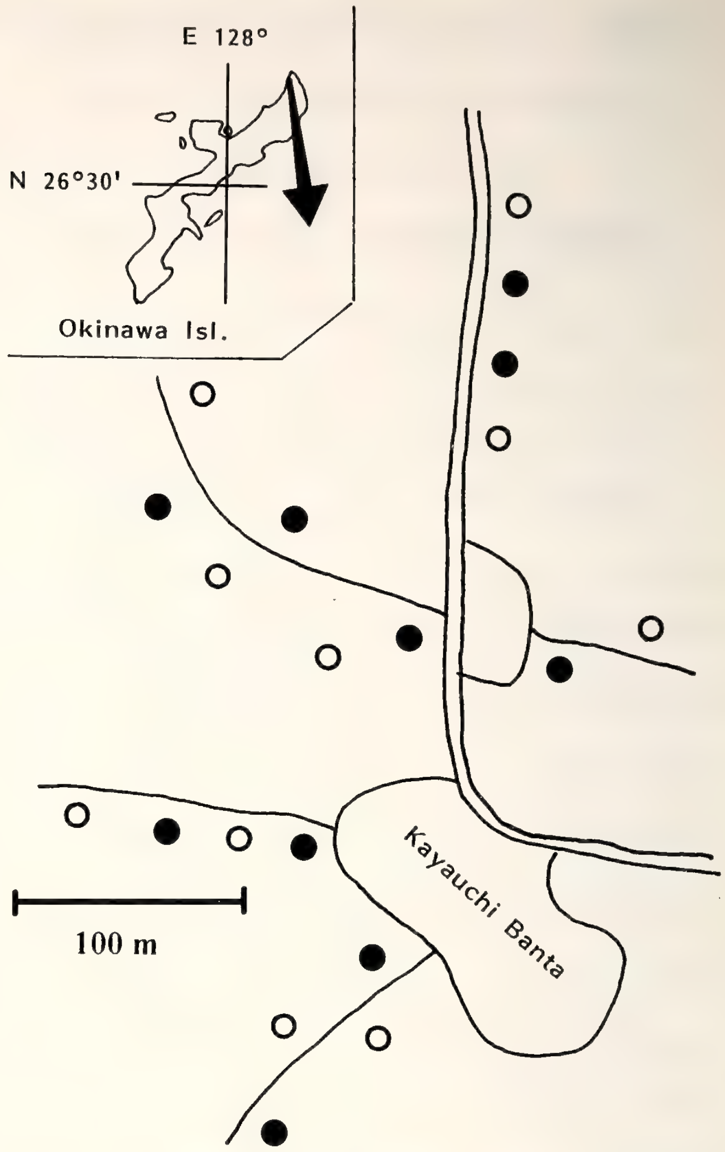

FIG. 3. Map of the study area at Ginama, Kunigami village, at the northernmost tip of Okinawa Island. Open and closed circles indicate the traps baited with a live mouse and those baited with mouse urine, respectively.

mouse urine and two blank traps were placed in the outdoor pen (Fig. 4), and nighttime observations were done using a night viewer between sunset and midnight. Again, on 20 August 1994, two traps baited with a live mouse and two blank traps were placed in the pen, and nighttime observations were done between sunset and midnight.

RESULTS AND DISCUSSION

During about six months of trapping, no habus were captured by the traps baited with mouse urine, whereas 14 habus were captured by the control traps baited with a live mouse. On the other hand, nine individuals of a colubrid snake, *Dinodon semicarinatum*, were

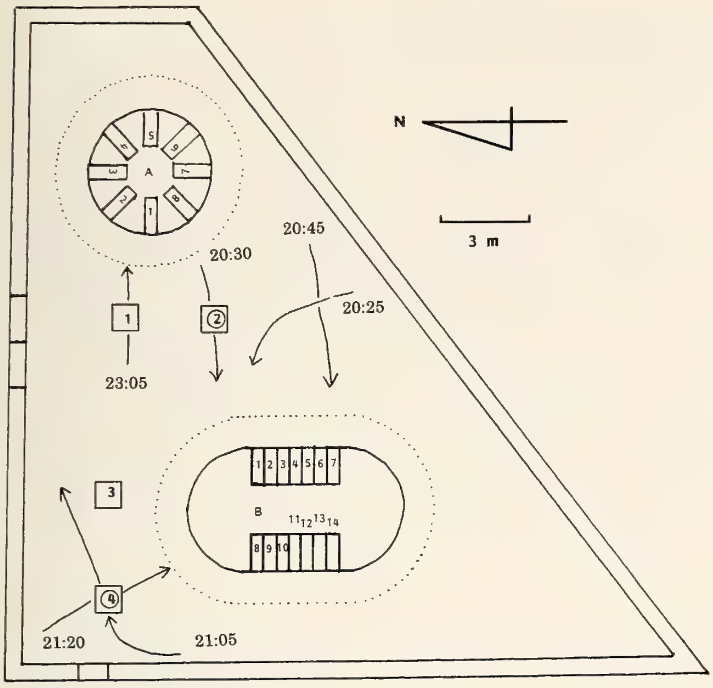

FIG. 4. Map of the outdoor pen at the habu study section, Okinawa Prefectural Institute of Health and Environment. The squares on the map indicate the position of the traps. The squares with a circle represent the traps baited with mouse urine. Circle-less squares are blank traps. Arrows indicate the movements of habus at night on 11 August 1994.

captured by both mouse urine and control traps.

On 11 August 1994, six habus were observed in the outdoor pen between sunset and midnight (Fig. 4). Three of the six habus approached the traps baited with mouse urine directly from a distance of 2-3 m distance. They all stopped crawling in front of the traps, and stuck their head into the traps. But, soon after that, they pulled out their head from the traps and crawled away. The next morning, no habus were found in the traps baited with mouse urine. During the same night, another habu approached a blank trap and climbed onto the trap, then crawled away without paying apparent attention to the trap. The next morning, no habus were found in the blank traps. On 20 August, three habus were observed between sunset and midnight. Two of them approached, directly from a distance of 2-3 m, a trap baited with a live mouse and immediately entered the trap. The next morning, two habus were found in this trap. No habus were captured by the blank traps.

Goris and Terashima (1973) demonstrated that the habu uses its pit organs (infrared receptors) to a high degree to detect and capture prey. Hattori et al. (1999) studied the foraging behavior of habus by counting tongue flicks and stated that odor cues alone are sufficient to release prey-searching behavior in habus. Nevertheless, habus were not captured by traps baited with only odor cues extracted from rats (Hattori et al., 1999) or by traps baited with only odor of mouse urine (the present study). These results suggest that habus can not be captured effectively by traps baited with odor cues alone. On the other hand, habus were captured by traps baited with a rat that was not visible (Hattori et al., 1999). The nighttime observations of the present study suggested that habus were attracted to the odor of mouse urine and approached the traps, but for some unknown reason, they did not enter the traps. Thus, it is suggested that habus can be attracted with chemical cues (odor) alone, but other stimuli such as thermal cues, vibrations, or chemical attractants more effective than urine and body odors are necessary to induce habus to enter the traps.

Mori et al. (1999) suggested that *D. semicarinatum* depends on chemical cues or vibrations to detect the nests of sea turtles in the beach. In the present study, as many *D. semicarinatum* were caught by the traps baited with mouse urine as by the traps baited with a live mouse (nine individuals). Therefore, it is apparent that foraging behavior of *D. semicarinatum* can be activated by the chemical cues alone as in the case of garter snakes (Chiszar et al., 1981; Mori et al., 1999).

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of Ogasawara (Bonin) Islands, South Japan. Bull. Herpetol. Soc. Japan 2000: 4-7. (in Japanese with English abstract)
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APPENDIX

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Catalogue numbers of specimens deposited in the zoological collection of Kyoto University Museum are preceded by KUZ. The other acronyms are those suggested by Leviton et al. (1985).

Geoemyda japonica: Okinawajima, Okinawa Pref., Japan, KUZ R36720, NSMT H02083-02086; Kumejima, Okinawa Pref., Japan, KUZ R36721, OMNH-R3334. G. spengleri: Vietnam, NSMT H9999, ...

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Other abbreviations, such as SVL (snout-vent length), Myr (million years), and MyrBP (million years before present), can also be used, but **define them at first usage**. Do not use symbols, ♂ and ♀, for male and female, respectively.

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Thermal Biology of an Iguanian Lizard, *Oplurus cuvieri cuvieri*, in a Tropical Dry Forest of Madagascar

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Abstract: Thermal characteristics of an Iguanian lizard, *Oplurus cuvieri cuvieri*, were examined in a tropical dry forest of northwestern Madagascar. This lizard is an arboreal, strictly diurnal sit-and-wait predator feeding mostly on insects. A field study was conducted from mid-September 1997 to mid-January 1998 and from late-September to mid-November 1998. Ambient air temperature significantly affected body temperature, but sex, snout-vent length, month, perching height, and posture of perching lizards did not show significant effects on body temperature. Effects of time and interaction between ambient temperature and time were significant in the first half of 1997 (dry season) and 1998, but not in the latter half of 1997. Overall body temperature was significantly correlated with ambient temperature, and slopes between them did not significantly deviate from one. However, slopes varied with time of day, and significant correlation between ambient and body temperatures diminished in the midday period. Body temperature was significantly higher than corresponding ambient temperature. In the rainy season, when ambient temperature decreases, the lizards seemed to regulate its body temperature to some extent by selecting hotter microhabitat (the ground). Collectively, although the study site is a relatively open, deciduous forest, where direct solar radiation for thermoregulation by basking is available, *O. c. cuvieri* exhibits intermediate features between thermoregulator and thermoconformer, having mixed characteristics of both types.

Key words: Madagascar; *Oplurus cuvieri cuvieri*; Iguania; Body temperature; Tropical dry forest

INTRODUCTION

In lizards thermal conditions can modify activity periods, which in turn, influence their life histories (Grant and Dunham, 1990). Thermal conditions can also interact with the physiological performance and influence the ecological distribution of species (Gorman and Hillman, 1977; Huey, 1982). Basically, a particular lizard species selects microhabitats that are both thermally and structurally suited to its physiological, morphological, and behavioral traits. For example, many lizards have a relatively narrow range of preferred body

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temperatures, which often corresponds to various physiological optima (Bennett, 1980; Huey, 1982). Similarly, lizards exhibit distinct preferences for particular substrates, perch heights, vegetation densities or other aspects of habitat structure depending on the thermal environment (Ruibal, 1961; Williams, 1983). Consequently, to compromise with various thermal demands and preference for structural habitats, lizards, especially diurnal species, exhibit various modes of behavioral thermoregulation.

Nonetheless, the vast majority of the available data on lizard thermoregulation are gleaned primarily from studies of temperate-zone species, where high daily fluctuation of ambient temperatures induces a lizard to devote considerable time and effort to thermoregulation if it attempts to maintain high and stable body temperatures (Avery, 1982). Contrary to temperate zones, in tropical forests, temporal and spatial variations in environmental temperatures may not have much influence on an animal's activities because the relatively benign environmental temperatures permit extensive thermoconformity (Avery, 1982; Shine and Madsen, 1996).

Most studies on thermal biology of lizards in tropical forests have been based on small diurnal species (e.g., Inger, 1959; Alcala and Brown, 1966), the group for which behavioral thermoregulation is likely to be most important because of their generally small body size combined with high diurnal thermal heterogeneity. The other tropical lizards that have been studied in this respect are those living in environments of low thermal heterogeneity such as densely vegetated forests (Avery, 1982; Rummery et al., 1994), where the opportunities for behavioral regulation of body temperatures are very limited. In such a habitat, many species have proved to be thermoconformers, that is, thermally passive animals, with little overt thermoregulatory behavior (Ruibal, 1961; Huey and Webster, 1976; Tanaka, 1986; Hertz et al., 1993). However, modes of thermoregulation are not always matched to the apparent thermal habitats. For example, Mori and Hikida (1993) showed the thermal passivity of a flying lizard, *Draco volans*, living in an open habitat of tropical Borneo, where direct solar radiation is readily available for lizards. In addition, an arboreal skink, *Apterygodon vittatus* inhabiting the same study area as the flying lizard, showed thermal conditions intermediate between baskers and nonbaskers (Mori et al., 1995). Similar intermediate or partial basking behavior has been reported in a few other lizard species in the tropics (Avery, 1982).

In the present paper, we investigated the thermal biology of an Iguanian lizard, *Oplurus cuvieri cuvieri*, living in a tropical dry deciduous forest of Madagascar. This species is a medium-sized (mean body mass in males and females are 147 g and 84 g, respectively; Randriamahazo, 2000), arboreal, and strictly diurnal lizard inhabiting relatively open forests. Although earlier studies of *O. c. cuvieri* suggested the presence of thermal preference of this lizard (Randriamahazo, 1998) and the absence of sexual differences in body temperature (Randriamahazo and Mori, 1999), morphological, behavioral, and environmental attributes that could affect the thermal biology of lizards were not adequately examined. Here, we characterized the thermal features of *O. c. cuvieri* by examining the effects of sex, size, month, time of day, perch height, and posture that are the potential source of variations in its body temperature.

Materials and Methods

Oplurus c. cuvieri is an endemic iguanian lizard of Madagascar (Glaw and Vences, 1994). This sit-and-wait insectivorous lizard is primarily arboreal, spending much of its time perching on tree trunks or fallen logs (Randriamahazo and Mori, 1999; Mori and Randriamahazo, 2002), although it occasionally moves on the ground for reproductive activity such as egg laying and territorial activity (Randriamahazo and Mori, 1999, 2001). Inactive lizards are found sheltering inside naturally sculptured hollows of stumps,

dead logs, and live trees (Blanc, 1977; Randriamahazo and Mori, 1999).

The study was conducted at the forest of Ampijoroa, Ankarafantsika Strict Nature Reserve (since 7 August 2002 part of Ankarafantsika, including Ampijoroa, is designated as a National Park), located in northwestern Madagascar (16°15'S and 46°48'E). The study site is a ca. 1 km×1 km dry deciduous forest having irregularly extended main paths (width=2–4 m) in it. A plot of approximately 16.6 ha (Jardin Botanique A) subdivided into ca. 5 m×5 m subplots by narrow trails (width=0.5–1.5 m) is present in the western area of the study site. All trails are human-made, and large trails are regularly maintained for tourism purposes. The elevation is approximately 200 m asl. The vegetation is dominated by three species of *Strichnos* trees generally 9–12 m in height (Razafy, 1987; Urano et al., 1994), and scattered shrubs and open areas with abundant leaf litter are present. The climate is mainly dominated by a rainy season from November to March and a dry season from April to October. In the dry season, most trees shed their leaves, making sunlight-rich, open understory and a forest floor with leaf litter. As the rainy season progresses, new leaves grow and the canopy cover increases, reducing the availability of solar radiation reaching the forest floor, although the main paths still provide sunlight-rich habitats (see a photograph in Mori et al, in press).

We collected lizards found outside their shelter trees mainly in the Jardin Botanique A throughout the daylight hours (between 0700 h and 1800 h) from 17 September 1997 to 15 January 1998 (hereafter referred to as year 1997) and from 29 September 1998 to 18 November 1998 (year 1998). Daily precipitation and daily maximum and minimum temperatures recorded at the study site during these periods are presented in Randriamahazo and Mori (2001). Within 10 s of capture of a lizard by noosing we measured its cloacal body temperature (BT; to the nearest 0.1 C) and ambient air temperature at 1 m off the ground in shade (AT) with a quick-reading mercury thermometer (Schultheis). Care was taken to prevent temperature from being influenced by handling, and all lizards requiring extensive efforts to capture were excluded from the following analyses. We recorded the time of day, predominant orientation of lizards' body (posture: horizontal, upward vertical, or downward vertical), and perch height of lizards above the ground. Based on preliminary observations, the vertical position with head facing downward may represent a readiness to chase after a prey item or an unwelcome intruder, whereas the head facing upward may indicate an alert posture related to basking or general survey of the home range area (Randriamahazo, unpublished data). We also measured the snout-vent length of lizards (SVL; to the nearest 1 mm). We assigned each captured lizard to adult males, adult females, or juveniles based on the criteria in Randriamahazo (2000). Lizards were marked individually by toe clipping, color painting, and passive integrated transponder (PIT) and released at the site of capture as soon as possible.

In the following analyses of BT, lizards that were perching stationary on substrate, such as trees, logs, and ground, were examined, and those actively moving, such as chasing, digging (for laying eggs), and running on the ground, were excluded to remove the possible temporary effects of reproductive activities on BT. Because some individuals were collected in both years, the following statistical analyses were conducted for each year separately to ensure the statistical independence of the data. In each year, each individual was contributed to only one dataset. The data used in the present study included some data used for the analysis of sexual differences of BT in Randriamahazo and Mori (1999). The difference of the dataset between these studies is due to the different objectives of the studies.

To determine factors that affect BT, we first conducted analysis of covariance (ANCOVA)

with AT and SVL as covariates, month and sex (male, female, juvenile) as factors, and BT as a dependent variable. Interactions between AT and month and between AT and sex were included in this ANCOVA model. Because, except for AT, no significant effects were detected (see Results), further ANCOVA was conducted with AT as covariate, time, perch height, and posture as factors, and BT as a dependent variable. Interaction between AT and time was included in this model. For this analysis both time and perch height were divided into five categories (time; 7–9, 9–11, 11–13, 13–15, and 15–18: perch height; 0 cm, 1–50 cm, 51–100 cm, 101–150 cm, and >150 cm). The data of 1997 were divided into two periods (data before 19 November, which is comparable to the study period of 1998 and approximately to the dry season, and those after 18 November) and analyzed separately. For comparisons of BT and AT among months and among time categories, one-factor analysis of variance (ANOVA) was used followed by multiple comparisons (Tukey-Kramer method). Regression analysis was made with Pearson correlation coefficient. Differences between AT and BT were tested using a paired t-test. ANCOVA was conducted using JMP 3.2.2 (1997). The other statistical analyses were conducted using StatView 5.0 (1998). All of the analyses were tested for statistical significance at the $P<0.05$ level.

RESULTS

In 1997 mean BT±SE of males, females, and juveniles was 35.4±0.4, 36.6±0.4, and 35.7±0.5, respectively, and in 1998 it was 37.3±0.7, 36.8±0.3, and 37.5±0.4, respectively. The first test with ANCOVA revealed that only AT significantly affected BT in both years although sex and interaction between AT and sex were close to a significant level in 1998 (Table 1). Hence, relationships between AT and BT were examined with all sexes and months combined. BT was significantly correlated with AT in both years (1997, $r=0.64$, $df=1, 204$, $F=139.4$, $P<0.0001$; 1998, $r=0.73$, $df=1, 125$, $F=140.7$, $P<0.0001$; Fig. 1). Slopes of regression lines between AT and BT were significantly different from 0 (1997, slope=0.898, $t=11.8$, $P<0.0001$; 1997, slope=0.997, $t=11.7$, $P<0.0001$), but their 95% confidence intervals overlapped with 1 in both years (1997, 0.748–1.048; 1998, 0.831–1.164). Overall mean AT and BT in 1997 were 31.8±0.2 and 35.8±0.2, respectively, and were 33.4±0.2 and 37.1±0.2 in 1998. BT was significantly higher than the corresponding AT in both years (1997, $df=205$, $t=20.8$, $P<0.0001$; 1998, $df=126$, $t=22.0$, $P<0.0001$).

To analyze the monthly variation in temperatures we used only the data of 1997, which were collected during a more extended period than the data of 1998. AT significantly varied among months ($df=4, 201$, $F=8.8$, $P<0.0001$; Fig. 2). Generally, AT was high in the dry

TABLE 1. Results of the initial test with analysis of covariance for body temperature of *Oplurus cuvieri cuvieri*.

	1997			1998		
Source of variation	df	F	P	df	F	P
Ambient temperature (AT)	1	110.18	<0.001*	1	9.50	0.003*
Snout-vent length	1	0.11	0.737	1	0.10	0.754
Sex	2	2.11	0.124	2	3.00	0.054
Month	4	1.98	0.100	2	0.15	0.861
AT×sex	2	1.59	0.206	2	3.01	0.053
AT×month	4	1.89	0.114	2	0.10	0.901

* Significant at $P<0.05$ level.

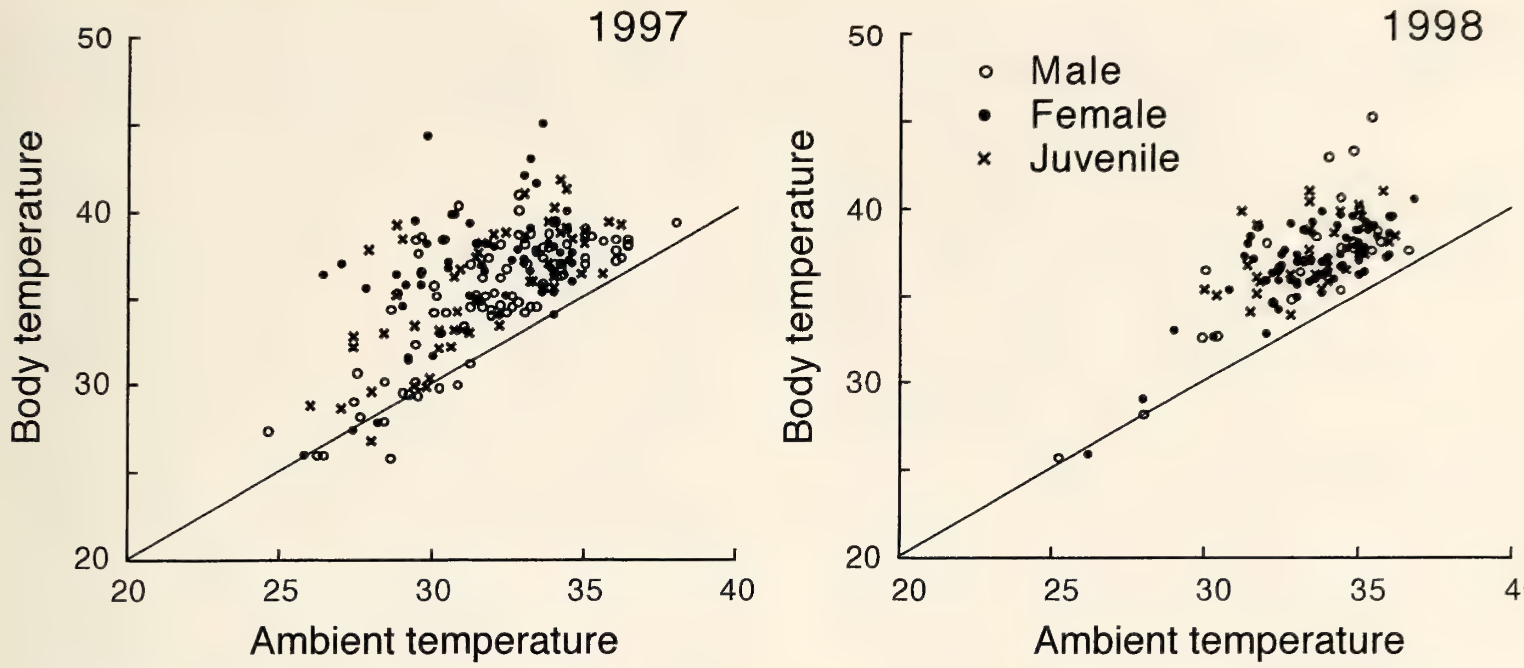

FIG. 1. Relationship between ambient air temperature and cloacal body temperature in males, females, and juveniles of *Oplurus cuvieri cuvieri* recorded in two study periods (1997 and 1998).

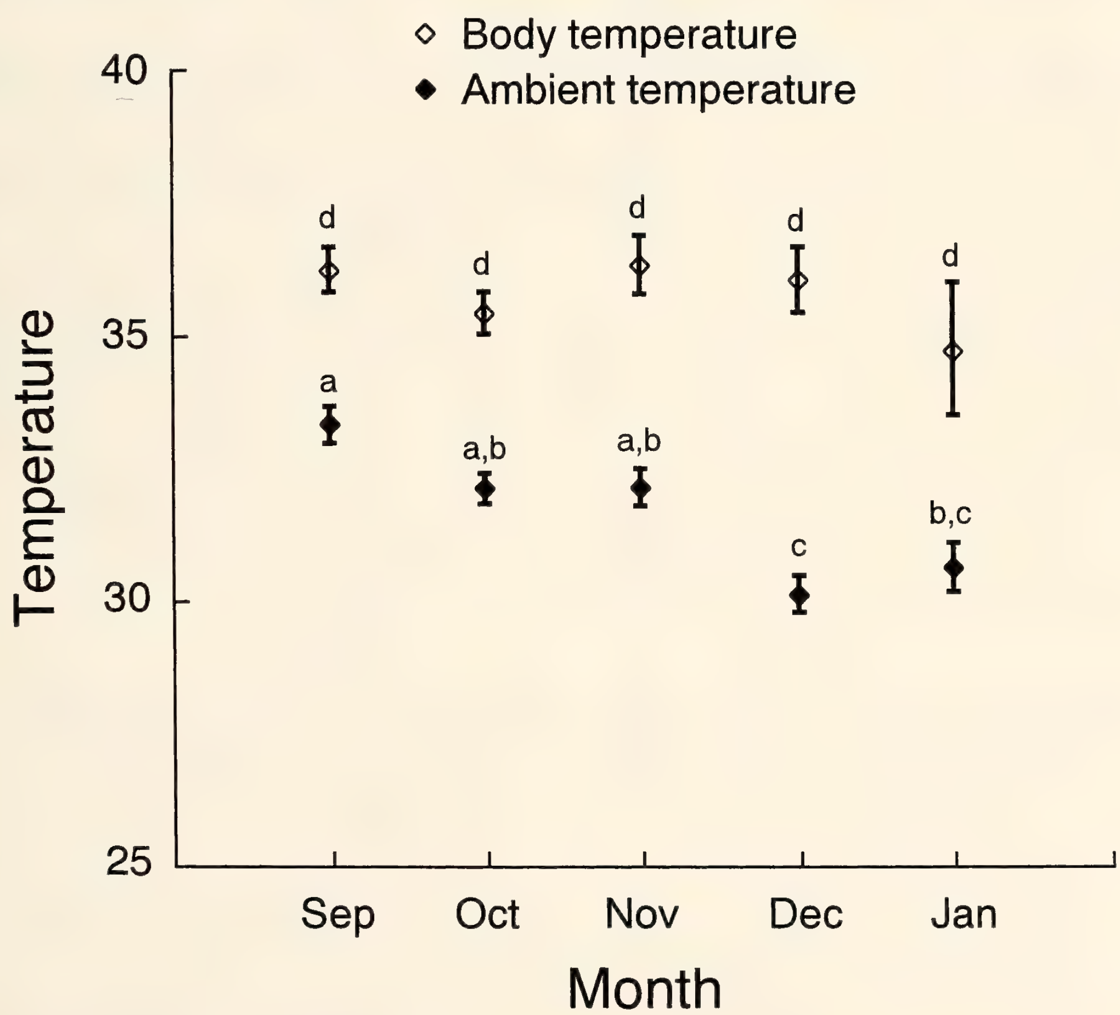

FIG. 2. Monthly variations in ambient air temperature and cloacal body temperature of *Oplurus cuvieri cuvieri*. Mean±1 SE are presented. Same lowercase letters above bars denote means that do not differ significantly based on pairwise multiple comparisons among months. Statistical comparisons between ambient and body temperatures for each month were not made.

season (September) and became lower in the rainy season (December and January). By contrast, BT did not vary significantly among months (df=4, 201, F=1.0, P=0.403; Fig. 2)

The second test with ANCOVA yielded similar results in 1998 and the first half of 1997, showing significant effects in AT, time, and interaction between AT and time (Table

2). On the other hand, in the second half of 1997 only AT showed significant effects (Table 2). The relationships between AT and BT separated by time are presented in Fig. 3. In 1998 and the first half period of 1997 BT was significantly correlated with AT in each time category except for 13–15. Comparisons of 95% confidence intervals of each slope indicated that the above significant interaction effects between AT and time in 1998 are attributable to the higher slope in the 7–9 time category than in the 15–18 time category, and those in the first half of 1997 are attributable to the higher slopes in 7–9 and 9–11 than in 11–13 and the higher slope in 9–11 than in 15–18 (Table 3). In the latter half of 1997 significant correlations between AT and BT were observed only in 11–13 and 15–18 time categories, in which slopes of regression lines were not significantly different from each other (Table 3).

In each year both AT and BT significantly varied among time (first half of 1997, AT, df=4, 125, F=22.9, P<0.0001, BT, df=4, 125, F=11.0, P<0.0001; second half of 1997, AT, df=4, 66, F=8.6, P<0.0001, BT, df=4, 66, F=2.9, P=0.028; 1998, AT, df=4, 120, F=21.0, P<0.0001, BT, df=4, 120, F=6.8, P<0.0001; Fig. 4). Generally, fluctuating patterns of BT paralleled those of AT. However, the results of pairwise multiple comparisons suggested that AT varied more extensively than BT in 1998 and the first half of 1997, which was reflected as the significant effects of

TABLE 2. Results of the second test with analysis of covariance for body temperature of *Oplurus cuvieri cuvieri*.

	1997 (before 19 Nov)			1997 (after 18 Nov)			1998		
Source of variation	df	F	P	df	F	P	df	F	P
Ambient temperature (AT)	1	80.42	<0.001*	1	16.86	<0.001*	1	70.45	<0.001*
Time	4	5.61	<0.001*	4	0.24	0.914	4	5.22	<0.001*
Perch height	4	0.89	0.475	4	2.06	0.099	4	1.83	0.128
Posture	2	1.91	0.153	2	0.39	0.677	2	2.86	0.062
AT×time	4	5.67	<0.001*	4	0.25	0.908	4	5.67	<0.001*

* Significant at P<0.05 level.

TABLE 3. Results of regression analysis between ambient temperature and body temperature of *Oplurus cuvieri cuvieri* for each time category. Figures in parentheses are 95% confidence intervals of slopes.

	1997 (before 19 Nov)				1997 (after 18 Nov)				1998			
Time of day	n	r	P	Slope	n	r	P	Slope	n	r	P	Slope
7–9	13	0.76	0.003*	1.85 (0.80–2.91)	8	0.42	0.299	—	6	0.94	0.006*	3.22 (1.56–4.87)
9–11	29	0.83	<0.001*	1.61 (1.19–2.04)	21	0.41	0.066	—	23	0.83	<0.001*	1.63 (1.14–2.13)
11–13	32	0.42	0.017*	0.43 (0.08–0.77)	19	0.61	0.005*	1.13 (0.39–1.88)	28	0.48	0.010*	0.98 (0.25–1.70)
13–15	11	0.32	0.331	—	8	0.62	0.103	—	21	0.27	0.243	—
15–18	45	0.83	<0.001*	0.81 (0.64–0.97)	15	0.61	0.015*	1.01 (0.23–1.80)	47	0.86	<0.001*	1.02 (0.84–1.20)

* Significant at P<0.05 level.

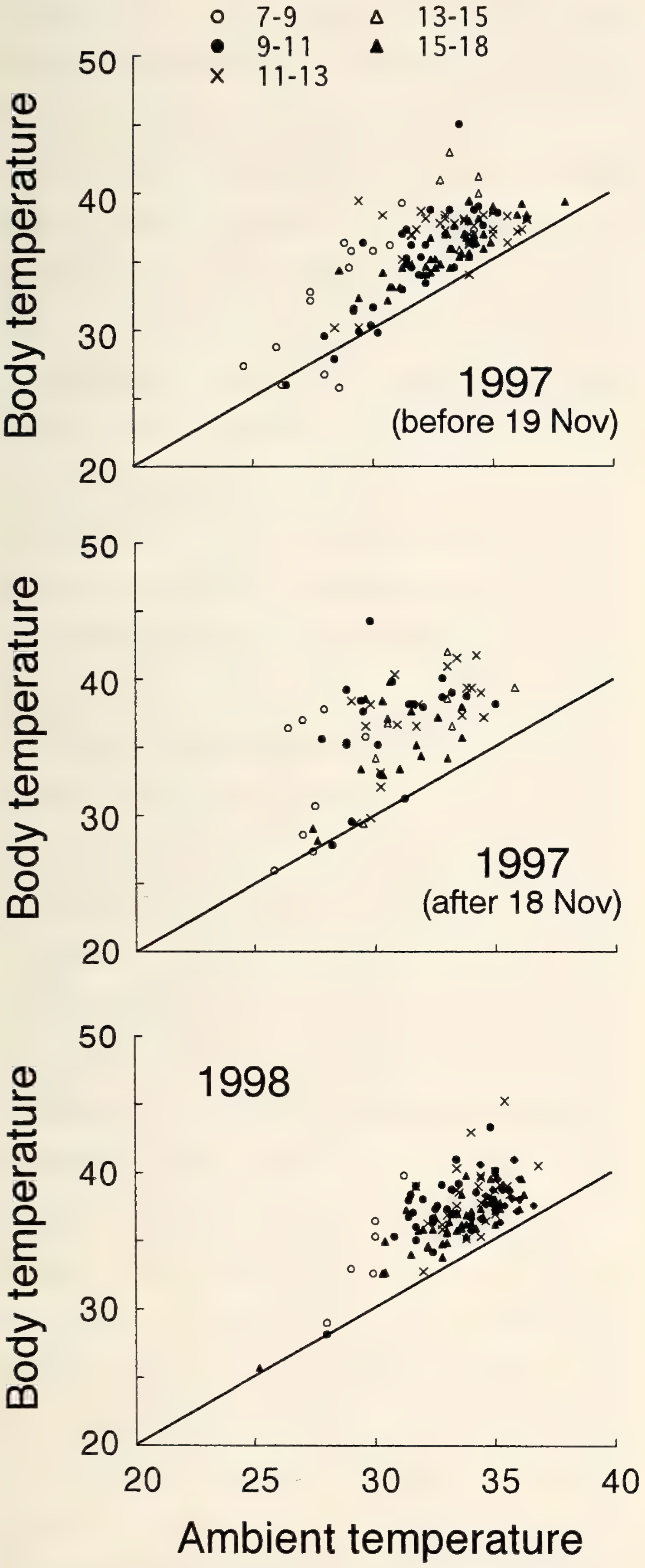

FIG. 3. Relationships between ambient air temperature and cloacal body temperature of *Oplurus cuvieri cuvieri* separately shown for five time categories.

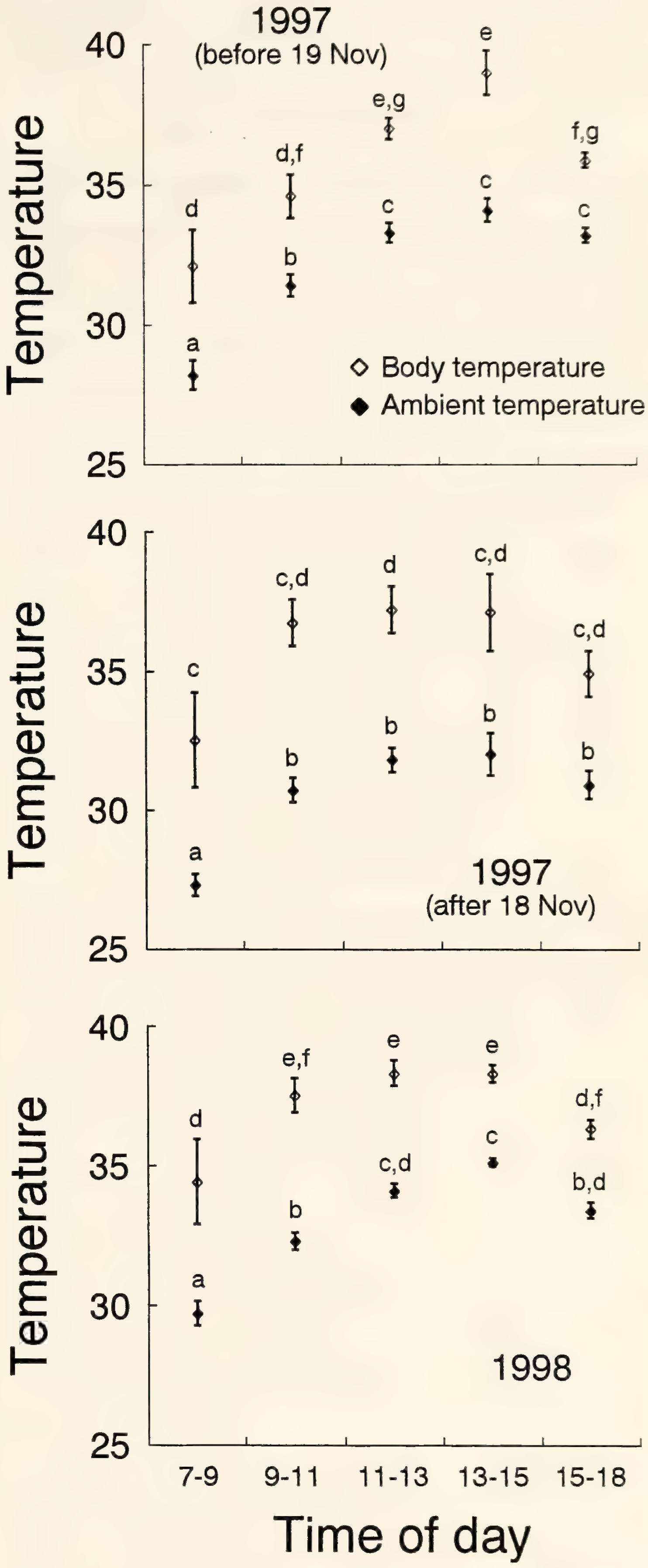

FIG. 4. Daily variations of ambient air temperature and cloacal body temperature of *Oplurus cuvieri cuvieri*. Means±1 SE are presented. Same lowercase letters above bars denote means that do not differ significantly based on pairwise multiple comparisons among time categories. Statistical comparisons between ambient and body temperatures for each time category were not made.

interaction between AT and time in the above ANCOVA,

Discussion

Unlike typical tropical rainforests, where closed vegetation precludes efficient thermoregulation by lizards, tropical dry forests such as the present study site (Ampijoroa) would provide lizards with much opportunity for strict themoregulation. In the Ampijoroa forest, predominant deciduous trees lose their leaves during a long dry season from April to October, and solar radiation for thermoregulation of lizards dwelling in the forest is available every day. As the rainy season begins around the end of October, new leaves grow and canopy cover increases. This physical barrier along with frequent cloudy or rainy climatic conditions reduce, both temporally and spatially, the amount of sunny spots available to the lizards on the forest floor and understory of the forest, especially after mid-rainy season around December to January. Nevertheless, the abundance of forest edges along complex artificial paths still provides open microhabitats for thermoregulation even during the rainy season. Therefore, it seems that in the Ampijoroa forest effective thermoregulation is potentially possible for lizards throughout the year.

The results of the present study suggest mixed features of thermoregulator and thermoconformer in *O. c. cuvieri*. The relatively broad range of BT, moderately significant correlation of BT with AT, and slopes of regression lines between AT and BT that do not significantly deviate from 1 are characteristics of a thermoconformer (Avery, 1982). The daily fluctuating pattern of BT corresponding to that of AT also suggests a thermally passive feature of the lizard. The absence of differences in BT between the sexes and between adults and juveniles may reflect the unimportance of thermoregulation in this lizard, although comparisons of BT between gravid and non-gravid females are desirable.

On the other hand, the relatively constant BT over months and less fluctuating daily BT compared to AT imply active thermoregulation by *O. c. cuvieri*. The relatively higher slopes of the regression lines between AT and BT in the morning and non-significant correlations between them during the midday in the dry season (1998 and the first half of 1997) also imply the occurrence of thermoregulatory behavior. Actually, the lizards often perched in sunlight: in 182 out of 413 sightings the lizard was perching in sunlight (Randriamahazo and Mori, 1999). The relatively constant monthly BT may have been maintained by the selection of sunlight-rich microhabitat, especially in December and January, when average AT and maximum daily air temperature of the study site decreased (see Randriamahazo and Mori, 2001 for the latter data). Comparisons of the perching height frequency among months of the first study period support this idea: *O. c. cuvieri* more frequently perched on the ground in the rainy season than in the dry season (September & October vs. November, $\chi^2=26.3$, $P<0.0001$; September & October vs. December & January, $\chi^2=39.3$, $P<0.0001$; Table 4). Based on a close examination of Fig. 1, BT seems to have a maximum set point temperature at approximately 40 C, suggesting thermoregulating behavior of the lizard to maintain its BT at a preferred maximum level. A few individuals showing extremely high BT (>41 C) may have been females just finished egg-laying on the sunny sandy trails (Randriamahazo and Mori, 2001) or males just after chasing or fighting with intruders (Randriamahazo and Mori, 1999) although we used only stationary individuals in the present analyses. Definitive determination of the

Table 4. Seasonal differences in the number of lizards for each perch height class.

Month	Perch Height (cm)				
	0	1–50	51–100	101–150	>150
Mid-Sep to Oct	3	44	34	8	5
Nov	19	16	20	3	2
Dec to mid-Jan	19	7	11	6	4

occurrence of thermoregulation requires tests of biophysical models as well as direct observations of thermoregulatory behaviors (Hertz et al., 1993).

Recently, Akani et al. (2002) stressed that one important contribution of thermoregulation in tropical reptiles is, as has been pointed out for lizards inhabiting hot desert environments (Avery, 1982), the avoidance of excessively high temperature, rather than the increase of body temperature to the preferred level. A study on the Madagascan day gecko, *Phelsuma madagascariensis kochi*, which was conducted in the same area of the Ampijoroa forest as in the present study, showed that the gecko is basically thermally passive, but it seems to decrease its BT when AT is higher than 30 C (Ikeuchi et al., in press). In contrast, BT of *O. c. cuvieri* was always higher than immediate AT except for a few cases when AT was lower than 30 C. Considering the ecological features common to these two syntopic species, that is, arboreal, diurnal, and insectivorous habits (Ikeuchi et al., in press; Mori et al., in press), it is interesting that the two species have quite different thermal propensities. A segregation of thermal propensity into baskers and nonbaskers has been reported in sympatric skink species in tropical forests (e.g., Inger, 1959; Fitch, 1973). The differences in thermal niche between *O. c. cuvieri* and *P. madagascariensis kochi* may enable their coexistence, because otherwise they have a very similar ecological niche. Thermal characteristics of other syntopic, diurnal, arboreal, insectivorous lizards, that is, chameleons of the genus *Furcifer*, as well as coexisting terrestrial lizards, such as *Mabuya elegans* (Scincidae) and *Zonosaurus laticaudatus* (Gerrhosauridae) (Mori et al., in press), are worth investigating to reveal the diversity of thermal strategies among syntopic lizards, which may be associated with the variability of costs and benefits of thermoregulation (Huey and Slatkin, 1976). Comparisons of their thermal strategies would clarify the mechanism of thermal niche segregation among tropical lizards co-inhabiting a dry deciduous forest (Randriamahazo, 1998).

Acknowledgments

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A New Species of *Cnemaspis* (Sauria: Gekkonidae) from Southern Thailand

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Abstract: A new species of *Cnemaspis* is described from Phuket Island, southern Thailand. The new species can be distinguished from congeners from Southeast Asia by the following combination of characters: SVL to 29.1 mm, two semi-circular supranasals separated by a single scale; three postnasals bordering nasal; four scale rows separate orbit from supralabials; posteriorly, each postmental bounded by three smooth, rounded, and juxtaposed scales; scattered spinose paravertebral rows of tubercles on dorsum; gular and pectoral scales unicarinate; abdominal scales not elongated, smooth; tail segmented, with enlarged flattened scales forming whorls, a single pair of spinose postcloacal spurs present; median subcaudals not enlarged, smooth; supralabials (to midorbit position) 6–7; infralabials 6–7; lamellae under toe IV 16–17; midventrals 26–32; and adult males lack preanal and femoral pores.

Key words: *Cnemaspis phuketensis*; new species; systematics; Reptilia; Sauria; Phuket Island; Thailand

INTRODUCTION

Four species of the gekkonid lizard genus *Cnemaspis* Strauch: *affinis* (Stoliczka, 1870) *chanthaburiensis* Bauer and Das, 1998, *kumpoli* Taylor, 1963, and *siamensis* (Smith, 1925), are known from within the political limits of Thailand (Chan-Ard et al., 1999). Two other species have been recorded from the country: Taylor (1963), in his review of the Thai herpetofauna, listed *C. kandiana* (Kelaart, 1852) (type locality: "Kandian hills, Ceylon" = hills of Kandy [or Mahanuwara], Central Province, Sri Lanka) and *C. mysoriensis* (Jerdon, 1853) (type locality: "Bangalore" [in Karnataka State, southwestern India]), from Thailand. The first species has also been recorded from mainland India (Annandale, 1909; Abdulali, 1955; Thakur, 1998; Thomas and Easa, 1997), the Andaman and Nicobar Islands (Smith, 1935), Thailand (Taylor, 1963; Cox et al., 1998), Sumatra (De Rooij, 1915; Rösler, 1981) and the Mentawai Archipelago (Boulenger, 1885, 1890). Bauer (2002) reported that the Western Ghats populations belong to a species distinct from *C. kandiana*, and Das (in press and unpubl.) will show that the

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Mentawai and Andaman and Nicobar species are not conspecific with the Sri Lankan species. Dring (1979), in his key to the genus for Southeast Asia included Taylor's *mysoriensis* in the synonymy of *kendallii*.

Cnemaspis is a speciose genus of gekkonids, and a large number of species have been described in recent years from southern and southeastern Asia (compare Kluge, 1993, 2001; Bauer, 2003; to Wermuth, 1966). We report here a new species of the genus from Phuket Island, southern Thailand. This species has been earlier reported as *C. kandiana* by Cox et al. (1998) and *Cnemaspis* sp. in Grossmann and Tillack (2000).

Materials and Methods

Specimens were hand-collected. The holotype was photographed in life and fixed in formalin, before storage in ethanol. The following measurements were taken ca. 18 months after collection with a Mitutoyo™ dial caliper (to the nearest 0.1 mm): snout-vent length (SVL; from tip of snout to vent); tail length (TL; from vent to tip of unregenerated tail); tail width (TW; measured at base of tail); head length (HL; distance between posterior edge of last supralabial and snout-tip); head width (HW; measured at angle of jaws); head depth (HD; maximum height of head, from occiput to throat); ear length (EL; greater ear length); forearm length (FA; distance between the palm and elbow); eye diameter (ED; greatest diameter of orbit); eye to nostril distance (E-N; distance between anteriormost point of eyes and nostrils); eye to snout distance (E-S; distance between anteriormost point of eyes and tip of snout); eye to ear distance (E-E; distance from anterior edge of ear opening to posterior corner of eyes); internarial distance (IN; distance between nares); and interorbital distance (IO; shortest distance between orbits). Scale counts and external observations of morphology were made using an Olympus SZX9 dissecting microscope. Notes on colour in life are from colour swatches of F. B. Smith (1975; 1981).

Comparative material examined is shown in the Appendix. Additional sources of information on character states include: Smith (1935), Nicholls (1949), Dring (1979), Das (1993), Manthey and Grossmann (1997), Das and Bauer (1998), Bauer and Das (1998), and Das and Grismer (2003). Catalogue numbers of specimens deposited in the Raffles Museum of Biodiversity Research, National University of Singapore, Singapore, are preceded by ZRC instead of USDZ as formerly proposed by Leviton et al. (1985). The other institutional abbreviations follow Leviton et al. (1985).

Cnemaspis phuketensis sp. nov.

Fig. 1

Holotype

ZRC 2.5212, Kathu Falls (07°55'N; 98°20'E), Changwat Phuket, Thailand, adult male, T. -M. Leong, collector, 1 September 2002.

Paratypes

ZRC 2.5214–18, paratopotypes, other data as for holotype, except collected 2 September 2002; ZRC 2.5233, Manik Falls, Changwat Phuket, Thailand, Tzi-Ming Leong, collector, 4 September 2002.

Diagnosis

A small species of *Cnemaspis* (SVL to 29.1 mm), distinguishable from conspecific species in showing the following combination of characters: two semi-circular supranasals separated by a single scale; three postnasals bordering nasal; four scale rows separate orbit from supralabials; posteriorly, each postmental bounded by three smooth, rounded and juxtaposed scales; scattered spinose paravertebral rows of tubercles on dorsum; gular and pectoral scales unicarinate; abdominal scales not elongated, smooth; tail segmented, with enlarged flattened scales forming whorls, a single pair of spinose postcloacal spurs present; median subcaudals not enlarged, smooth; supralabials (to midorbit position) 6–7; infralabials 6–7; lamellae under toe IV 16–17; midventrals 26–32; and adult males lack preanal and femoral pores.

FIG. 1. The holotype of *Cnemaspis phuketensis* sp. nov. (ZRC 2.5212) in life.

Description of holotype

A small species of *Cnemaspis* (snout-vent length 29.0 mm); snout elongate, large (HL/SVL ratio 0.18), narrow (HW/SVL ratio 0.16), depressed (HD/HL ratio 0.66), distinct from neck; lores sloping and interorbital region flattened; snout long (E-S/HW ratio 0.87), longer than eye diameter (ED/E-S ratio 0.43); scales on snout and forehead warty, under magnification revealed as tubercles that are raised towards posterior of each scale; scales on snout larger than those on occipital region; eye large (ED/HL ratio 0.32); 'extra-brillar fringes' (of Underwood, 1954) indistinct; pupil round; elongated supraciliaries on top half of orbit; ear-opening deep, slit-like, its greatest diameter vertically, fairly narrow (EL/E-S ratio 0.13); eye to ear distance greater than diameter of eyes (E-E/ED ratio 1.59); no ridge of tubercles along mandible or from posterior of orbit to postero-venter of tympanum, or from antero-dorsum of tympanum to nape; rostral completely divided by a simple rostral groove which meets anterior of snout; rostral less than half as deep as wide (rostral width= 1.3 mm/rostral depth=0.5 mm; width/depth ratio 2.6); contacted posteriorly by two nostrils and two semi-circular supranasals that are separated by a single scale; ventro-posteriorly, rostral is in contact with supralabial I; nostrils oval, situated within nasals, and oriented laterally; nasals reduced, in broad contact with supralabial I; anterior nasal larger than posterior nasal; three postnasals bound nasal; four scale rows separate orbit from supralabials; mentals subtriangular, deeper than wide, paired postmentals that are semicircular, smaller than mental and separated by a single scale; posteriorly, each postmental is bounded by three smooth, rounded and juxtaposed scales; tongue narrowly elongate, with a weak median cleft.

Body slender, elongate (A-G/SVL ratio 0.51); scale size does not decrease dorsally after thorax; ventrally, scales do not decrease in size from chin region to gular, pectoral and abdominal regions; scales on dorsum at midbody unicarinate, smaller than those of ventrum at same level; scales along vertebral region not differentiable from adjacent scales; scattered spinose tubercles on paravertebral

region not arranged in rows; pectoral and abdominal scales not elongate, smooth; spinous processes on lateral surface of body; scales on manus and pes smooth, rounded; scales on inner surface of forearm, distal aspect of upper arm, dorsal surface of thighs, tibia; upper arm and forearm unicarinate.

Forelimbs moderately long, slender; hindlimbs relatively short; tibia short (TBL/SVL ratio 0.18); no shield-like subtibial scales; digits elongate, all bearing claws that are slightly recurved; subdigital scansors entire, unnotched; no fragmented basal lamellae, six enlarged scansors at base of digits, which are more than twice width of other scansors; lamellae under digit IV of pes 16 (including the enlarged basal ones); interdigital webbing absent; relative length of digits (finger): 3>2>4>5>1; (toe): 3>4>5>2>1.

Original tail long, tip missing, preserved portion of tail longer than snout-vent length (TL/SVL ratio 1.21); tail tip blunt, tail base swollen, segmented, with enlarged flattened scales with a median keel forming whorls, a single pair of spinose postcloacal spur present; tail with a distinct pair of furrow laterally; median subcaudals not enlarged, smooth; scales on postanal region and at proximal part of tail base and on rest of subcaudals smooth; hemipeneal swelling at tail base, preanal depression present.

Scutellation.

See Table 1.

Colouration (in life)

Dorsum olive (# 30), with sinuous, dark grayish brown (# 20) markings on nape, axilla, torso and inguinal regions, labials dark-barred; spinose tubercles on flanks chamois (# 123D); limbs and tail dark-banded; a dark grayish brown (# 20) canthal stripe, commencing from snout, traversing the orbit region and extending to axilla; throat and undersurface of tail with fine hair brown (# 119A) mottlings; rest of venter unpatterned cream; iris buff yellow (# 53); pupil black.

Measurements (in mm).

See Table 2.

Sexual dimorphism

ZRC 2.5212, 2.5214–17 are adult males (presence of hemipeneal swelling at tail base, with preanal depression; ZRC 2.5218 and 2.5233 are adult females (lack of hemipeneal swelling or preanal depression). Preanal and femoral pores are absent in males. Females lack obvious endolymphatic sacs that are visible externally in many adult female geckos.

Etymology

Latin implying an inhabitant of Phuket Island, in southern Thailand.

TABLE 1. Squamation data of the holotype (ZRC 2.5212) and paratypes of *Cnemaspis phuketensis* sp. nov. See text for details. Abbreviations: +=present; −=absent; M=male; F=female; SL=supralabial; IL=infralabial; IO=interorbital; T4=lamellae under toe IV of pes; MV=midventral; PA=preanal depression

	Specimen						
Character	ZRC 2.5212	ZRC 2.5214	ZRC 2.5215	ZRC 2.5216	ZRC 2.5217	ZRC 2.5218	ZRC 2.5233
SL (MO)	6	6	7	7	7	6	7
IL	6	6	7	6	7	7	7
IO	9	7	7	7	8	7	7
T4	16	16	17	16	17	17	17
MV	30	28	26	30	32	32	32
Sex	M	M	M	M	M	F	F
PA	+	+	+	+	+	−	−

TABLE 2. Measurements (in mm) of the holotype (ZRC 2.5212) and paratypes of *Cnemaspis phuketensis* sp. nov. See text for details. Abbreviations: O=original tail; R=regenerated tail; SVL=snout-vent length; TL=tail length; FA=forearm length; TBL=tibia length; A-G=axilla to groin distance; HL=head length; HW=head width; HD=head depth; ED=eye diameter; E-E=eye to ear distance; E-S=eye to snout distance; E-N=eye to nostril distance; IO=interorbital distance; EL=ear length; and IN=internarial distance.

	Specimen						
Character	ZRC 2.5212	ZRC 2.5214	ZRC 2.5215	ZRC 2.5216	ZRC 2.5217	ZRC 2.5218	ZRC 2.5233
SVL	29.0	27.1	27.7	26.1	28.4	29.1	26.8
TL	35.0	28.2	21.1	34.2	21.3	33.6	32.5
FA	5.5	5.0	4.8	4.1	4.3	4.6	4.5
TBL	5.2	5.0	5.5	5.5	5.4	5.5	5.3
A-G	14.9	14.1	12.2	12.9	13.4	12.8	12.1
HL	5.3	4.7	5.3	5.0	5.1	5.7	4.8
HW	4.6	4.6	5.0	4.3	4.7	5.3	4.5
HD	3.5	3.1	4.1	3.0	3.2	3.6	3.2
ED	1.7	1.5	1.8	1.5	1.7	1.5	1.5
E-E	2.7	2.5	2.2	2.2	2.4	2.5	1.9
E-S	4.0	3.2	3.5	3.0	3.7	3.5	3.3
E-N	2.8	2.3	2.8	2.7	2.6	2.8	2.5
IO	3.0	2.4	2.7	2.2	2.7	2.8	2.2
EL	0.5	0.3	0.4	0.5	0.5	0.5	0.5
IN	0.9	1.1	1.1	1.0	1.1	1.1	1.0
Tail	O	O	R	O	R	O	O

Natural history notes

The holotype was taken from a leaf of an herb, 5 cm above substrate; the paratypes from either earth banks beside a small stream or from tree trunks at chest level, during the day. Leong et al. (2003) recorded the following additional saurian species as sympatric: *Draco taenioptera* Günther, 1861 (at Kathu Falls), and *Cyrtodactylus oldhami* (Theobald, 1876) (at both Kathu and Manik Falls).

Comparisons

The new species from Phuket, Thailand, is compared with congeners from Thailand. Only opposing suites of characters are listed. *Cnemaspis affinis* (Stoliczka, 1870), distribution: southern Thailand and Peninsular Malaysia: SVL to 48.0 mm; a dark marking at axilla; no enlarged tubercles on tail; and dorsal surface with five transverse yellow bands; *C. chanthaburiensis* Bauer & Das, 1998, distribution: Chanthaburi Province, Thailand: SVL to 41.0 mm; a ridge of tubercles border anterior margin of ears and another from ear to nape; a series of white paravertebral markings between nape and tail; and belly mottled light brown; *C. kumpoli* (Taylor, 1963), distribution: Trang in southern Thailand: SVL to 52.0 mm; supralabials 11; posterior supralabials with longitudinal keels; and three postcloacal spurs; and *C. siamensis* (Smith, 1925), distribution, Thailand and northern Peninsular Malaysia: SVL to 39.7 mm; forehead with keeled scales; supralabials 9–11; infralabials 8–10; paravertebral tubercles in 12 or 14 rows; and ventrals and subcaudals tricarinate.

The new Thai species is next compared with other south-east Asia congeners. *C. argus* Dring, 1979, distribution: Gunung Lawit in northern Terengganu, Malay Peninsula: SVL to 65.3 mm; fourth and fifth fingers subequal; supralabial VIII in midorbital position; and

yellow radiating lines from the orbits; *C. boulengerii* Strauch, 1887, distribution: Con Son Island, Vietnam: SVL to 66.0 mm; supralabials 8–10; infralabials 7–8; a series of 6–7 shield-like subtibial scales; subcaudal scales almost as wide as tail, and large black nuchal and shoulder spots; *C. dringi* Das & Bauer, 1998, distribution: Kapit District, central Sarawak, East Malaysia (Borneo): SVL to 45.5 mm; five postnasals; postcloacal spurs absent; toe IV lamellae 20; flanks with distinct white patches; and ventrals heavily pigmented; *C. flavolineata* (Nicholls, 1949), distribution, the northern Malay Peninsula: SVL to 46.7 mm; yellow line along back; paired, elongate postmentals; and lamellae under toe IV 28; *C. gordongekkoi* Das, 1993, distribution, Lombok Island, Lesser Sundas, Indonesia: SVL to 73.0 mm; lamellae under toe IV 22–23; supralabials at midorbital position 9; infralabials 10; interorbitals 20, and throat scales smooth; *C. kendallii* (Gray, 1845), distribution: Borneo, the Riau Archipelago and peninsular Malaysia: SVL to 58.0 mm; tubercles on dorsum keeled; postnasals six; postmentals bounded by 4–5 scales posteriorly; two rows of tubercles run posteriorly from orbit towards tympanum; and median subcaudal scales raised; *C. nigridia* (Smith, 1925), distribution, Bau and Gunung Gading, western Sarawak, East Malaysia (Borneo) as well as Natuna Island: SVL to 69.8 mm; supranasals in wide contact; postmentals bounded by four scales posteriorly; postnasals five; two rows of tubercles run posteriorly from orbit towards tympanum; and tail without dark bands; and *C. timoriensis* (Duméril & Bibron, 1836), distribution, Timor, without specific information, and may be either West Timor, Republic of Indonesia, or the newly-independent nation of East Timor: SVL to 35 mm; dorsum with no enlarged tubercles; supralabials five; lamellae under toe IV 12; and dorsal surface reddish-brown, with a series of brown paravertebral spots.

Two further species of the genus have been recently described from the Seribuat Archipelago, off the east coast of Peninsular Malaysia (Das and Grismer, 2003). One of these, *Cnemaspis limi* from Pulau Tioman, Pahang State, can be differentiated from the new Thai species in being significantly larger-SVL to 88.2 mm; supranasals in contact; supralabials 11–14; and scales on forearm smooth. The second Seribuat species, *C. baueri*, is from Pulau Aur, Johor State, and differs from the new species in being much larger, SVL to 64.9 mm; fourth toe longer than fifth; lamellae under toe IV 26–27; supralabials 11–13; scales on forearm smooth; and caudal bands absent.

Affinities of the new Thai *Cnemaspis* remain unknown, for lack of a phylogenetic hypotheses for the genus. Several distinct and apparently unrelated species, once allocated to *C. kandiana*, are now known, the association with this Sri Lankan endemic is for sharing spinose flanks, comprising tuberculate scales, keeled gular scales, and the presence of preanal and femoral scales.

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APPENDIX

Comparative material examined

Cnemaspis affinis (Stoliczka, 1870): ZSI 5964 (holotype), ZRC 2.1098, "Penang" (=Pulau Pinang, West Malaysia); ZMA 11987, Pinang, West Malaysia; ZRC 2.4858, Moon Gate, Pulau Pinang, West Malaysia; Maxwell's Hill, Perak, West Malaysia (ZRC 2.1100; 2.1099).

Cnemaspis boulengerii Strauch, 1887: CAS 73745 (paratype of *Gonatodes glaucus* Smith, 1920), MCZ 39014–23, "Pulo Condore" (=Con Dao), Vietnam.

Cnemaspis chanthaburiensis Bauer & Das, 1998: FMNH 215979 (holotype), "Khao Soi Daouw (Dao) Wildlife Sanctuary, Pongnomron (Pong Nam Ron), Chantaburi (Chanthaburi) Province, Thailand (approximately 13°00'N, 102°05'E)"; BMNH 1917.5.14.4 (paratype), "Chantaburi (Chanthaburi Province), Siam (Thailand)"; FMNH 191479 (paratype), "Khao Soi Dao Tai, Pong Nam Ron, Chantaburi (Chanthaburi) Province, Thailand (approximately 13°00'N, 102°05'E), 850 m."; FMNH 215978 (paratype), "Khao Khiew (Khieo) Wildlife Sanctuary, Chon Buri Province, Thailand (approximately 13°14'N, 101°08'E)"; FMNH 215980 (paratype), "Amphoe Muang, Suan Kaset, Chantaburi (Chanthaburi) Province, Thailand (approximately 123°6'N, 102°09'E)".

Cnemaspis gordongekkoi Das, 1993: ZRC 2.3380 and ZRC 2.3381 (holotype and paratype), "..vicinity of Sendanggila Falls, *circa* 0.5 km south of Senaru village, Lombok, Nusa Tenggara District, Republic of Indonesia (8°45'S, 116°30'E)".

Cnemaspis dringi Das & Bauer, 1998: FMNH 148588 (holotype), "Labang Camp (03°20'N; 113°29'E), Bintulu District, Fourth Division, Sarawak, East Malaysia, Borneo"; FMNH 221478 (paratype), "Sungai Segaham (02°44'N; 113°53'E), Belaga District, Seventh Division, Sarawak, East Malaysia".

Cnemaspis kandiana (Kelaart, 1852): BMNH 60.3.17.1066, 80.2.2.119, 53.4.1.1 (three syntypes), "Kandian hills, Ceylon" (=hills of Kandy [or Mahanuwara], 07°15'N; 80°40'E, Central Province, Sri Lanka); MCZ 4138, 26719, "Ceylon" (=Sri Lanka); ZSI 5971 (holotype of *Gymnodactylus Humei* Theobald, 1876), "Kandy" (see comments above); MSNG 8764 (four specimens), "Ceylon" (=Sri Lanka).

Cnemaspis kendallii (Gray, 1845): BMNH XXII.92a (lectotype, designated by Dring, 1979), "Borneo"; FMNH 223201, MCZ 157158–59. Bako National Park, Sarawak, East Malaysia (Borneo); FMNH 223201; MCZ 157158–59. Bidi, Sarawak, East Malaysia (Borneo); FMNH 184424. Bukit Lanjan, Selangor, West Malaysia; BMNH 1902.12.12. 12. Bidi, Sarawak, East Malaysia (Borneo); Bau, Sarawak, East Malaysia (Borneo); BMNH 1911.1.20.7–9. Bau, Sarawak, East Malaysia (Borneo); BPBM 7494, Alag Sungei Ayer, Pulau Tioman, Pahang, West Malaysia; ZRC 2.1101. Jerantut, Pahang, West Malaysia; ZRC 2.1102, Gunung Rokan, Pulau Tioman, Pahang, West Malaysia; ZRC 2.1103. Sedagong, Pulau Tioman, Pahang, West Malaysia; ZRC 2.1109–10. Pulau Siantan, Anamba, Riao Archipelago, Indonesia; ZRC 2.1112–13. Sungei Ulu, Great Natuna, Riao Archipelago, Indonesia; ZRC 2.3014. Bukit Timah, Singapore; ZRC 2.3015. Gunung Ladang, Melaka, West Malaysia; USNM 26573. Pulau Bunoa, Tambelan Islands, Indonesia; USNM 26555. St.

Barbe Island, at present Pulau Pedjantan, Indonesia; USNM 26547–49. Bunguran, Natunas, Riao Archipelago, Indonesia; USNM 28145. Pulau Lingung, Natuna, Riao Archipelago, Indonesia; USNM 28149. Sirhassen, Natuna, Riao Archipelago, Indonesia; also UF 78463 and ZSI 14767 and 19637, from "Borneo".

Cnemaspis nigridia (Smith, 1925): BMNH 1946.8.22.90 (formerly BMNH 1925.9.1.8; holotype), MCZ 39024 and ZRC 2.1114–115, "Mt. Gadin" (=Gunung Gading, 01°44'N; 109°50'E, Sarawak, East Malaysia; Borneo); MCZ 15250, Lundu, Sarawak, East Malaysia; BMNH 1925.9.1.9–10, Gunung Pueh, Sarawak, East Malaysia.

Cnemaspis siamensis (Smith, 1925): MCZ 39025, Maprit, Patiyu, peninsular Thailand; MCZ 39694, Klong Bang Lai, peninsular Thailand.

Cnemaspis limi Das & Grismer, 2003: ZRC 2.5289 (holotype); ZRC 2.5290 (paratype). "Gua Tengkuk Air, Gunung Kajang (02°50'N; 104°09'E), Pulau Tioman, Pahang, West Malaysia, altitude 980 m"; ZRC 2.3504–06 (three paratypes). Tekek-Juara trail (02°52'N; 104°12'E), Pulau Tioman, Pahang, West Malaysia.

Cnemaspis baueri Das & Grismer, 2003: ZRC 2.5291 (holotype); ZRC 2.5292–99 (eight paratopotypes). Kampung Berhala (02°27'N; 104°30E), Pulau Aur, Johor, West Malaysia.

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Field Observations on a Highly Endangered Snake, *Opisthotropis kikuzatoi* (Squamata: Colubridae), Endemic to Kumejima Island, Japan

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Abstract: The Kikuzato's brook snake, *Opisthotropis kikuzatoi*, is a highly endangered aquatic or semiaquatic species endemic to Kumejima Island of the Okinawa Group, Ryukyu Archipelago. Field studies were carried out for some ecological aspects of this species by visiting its habitat almost every month from April 1996 to March 1997. The results demonstrate that the snake is active almost throughout the year. It is also suggested that the snake tends to be diurnal in the warmer season, and nocturnal in the cooler season. Observations on a case of autonomous emergence onto the land, very slow growth, and predation on small crabs, are also provided.

Key words: *Opisthotropis kikuzatoi*; Field census; Activity pattern; Body temperature; Kumejima Island

INTRODUCTION

The Kikuzato's brook snake *Opisthotropis kikuzatoi*, is a small colubrid species endemic to Kumejima Island of the Okinawa Group, Ryukyu Archipelago. Since its original description by Okada and Takara (1958: as a member of the genus *Liopeltis*), no more than ten individuals have been known to science (five preserved specimens and other five individuals examined and released: Toyama, 1983; Ota and Mori, 1985; OPBE, 1993). Such an extremely limited distribution and rarity of observations, along with apparent progression in the reduction and fragmentation of its putative habitat (i.e., running waters surrounded by broadleaf evergreen forest: see below), led Environment Agency of Japan assign *O. kikuzatoi* to the highest Red List Category (IA) as one of the two most critically endangered reptiles of Japan (Matsui, 1991; Ota, 2000). This snake is also protected by laws of both the National Government of Japan and the Prefectural Government of Okinawa (Ota, 2000).

To the present, very few ecological data, largely obtained through captive observations, are available for *O. kikuzatoi* (Ota and Mori, 1985; Mori and Nakachi, 1994: but see OPBE [1993] for a few field observations). Such a scarcity of field data obviously makes it difficult to develop effective conservation measures for this critically endangered species. Recently I obtained some ecological data for *O. kikuzatoi* through a series of field surveys as below.

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Materials and Methods

Of the ten individuals of *Opisthotropis kikuzatoi* hitherto reported, all but one were found under the water in streams and brooks running on the floor of the evergreen broadleaf forest (Toyama, 1983; Ota and Mori, 1985; OPBE, 1993). The other individual was also found in a stream in the forest, but with almost no surface water because of the summer drought (OPBE, 1993). I thus fixed a census course along a middle 350 m portion of one brook, which was located on the border between the evergreen broadleaf forest on the mountain side and open grassy vegetation on the lowland side.

During the period from April 1996 to March 1997, I, together with one or two field assistants, visited the site every month but October, staying there for 24 hours (see Appendix 1 for further details). In each visit, census was carried out every hour from 0600 to 2000 and every second hour from 2000 to 0600 by slowly (ca 10 m/min) walking on the lowland side along the stream, searching for the snakes both in the water and on the banks. For the census at night, a flashlight was used as an aid. At the beginning of each census, water temperature (measured 10 mm below the surface), air temperature (measured in shade at 1 m above the ground), and water depth were measured at a fixed standard point (henceforth referred to as SWT, SAT, and SWD, respectively).

When a snake was found, its behavior was continuously observed as long as possible. However, when its escape into vegetation or other obstacles was likely, the snake was captured immediately. After capture, its body temperature (BT) was measured within 30 sec by inserting the sensor probe of an electronic thermometer (thermistor) into the cloaca. Water temperature (WT: 10 mm below the surface) and air temperature (AT: in shade, 1 m above the ground) were also measured at the point where the snake was located at capture. All temperature measurements (including SWT, SAT and SWD) were taken to the nearest 0.1 C using thermistor (Takara Digimulti D611) equipped with glass sensor probe.

Each snake captured was sexed by everting the hemipenes or by examining the external shape of the tail base, and then was subjected to examination for snout-vent length (SVL), tail length (TL), body weight (BW), and ventral (VT, sensu Dowling, 1951) and subcaudal counts (SC). Of these, SVL and TL were measured to the nearest mm with a tape measure, and BW to the nearest 0.1 g with a portable electronic balance (Shimazu AXEWB-35). Stomach contents were examined by palpation and forced regurgitation. For females, oviductal eggs were also examined by palpation. Finally the snake was individually marked by ventral clipping and released at the point where it was captured. The whole process was completed within 15 min so as not to exhaust the snake through handling.

Even while a captured snake was examined, the census was also continued as scheduled by a field assistant. Animals other than *O. kikuzatoi* encountered during the census were also recorded.

Results

Opisthotropis kikuzatoi was found 11 times during the study period. Snakes were invariably active, moving on the bottom of the stream, when they were first located. In a finding in June, the snake successfully escaped by suddenly emerging onto the forest side bank and quickly entering dense shrubbery. Of the remaining ten findings in which snakes were captured for further examination (see above), one in the early afternoon of 26 August and another after midnight of March 23 involved the same individual (Fig. 1). Snakes involved in the remaining eight captures included no recaptures.

Of the nine individuals examined, four (captured in July, August and March, September, and December) were males, and the remaining five (April, July, September, November, and December) were females. Data for quantitative external characters of these specimens were combined with data from previous

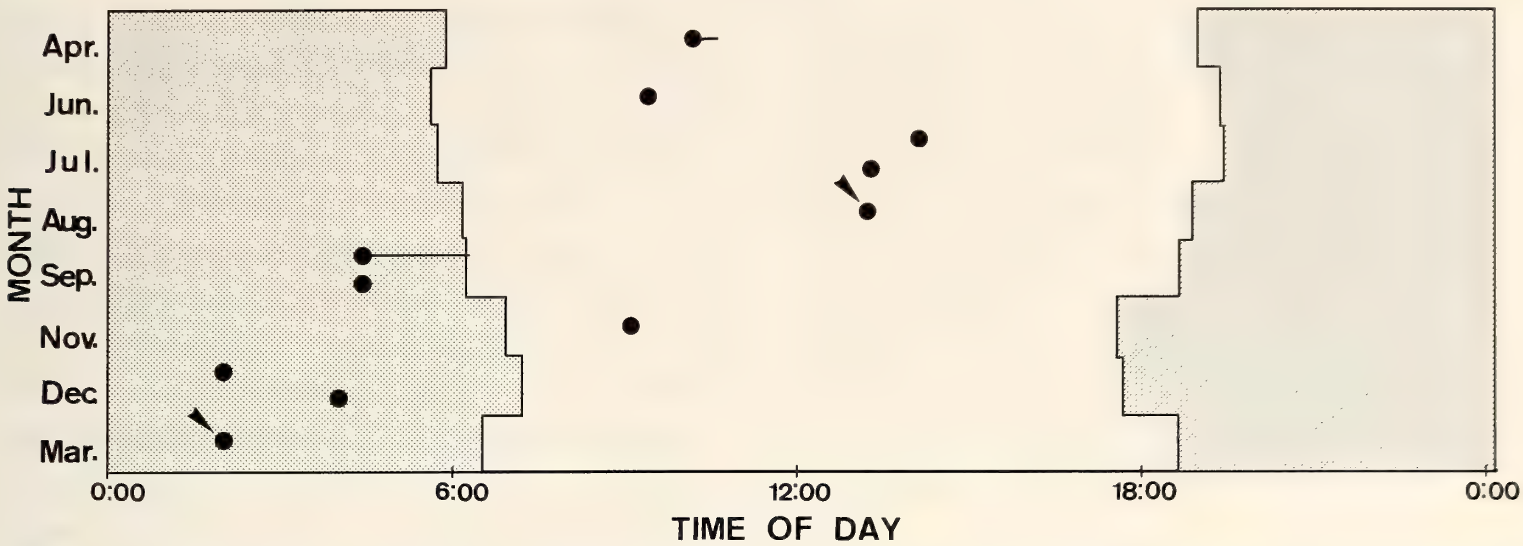

FIG. 1. Times of day at which *Opisthotropis kikuzatoi* were found. Stippled area represents the extent of night (i.e., from sunset to the next sunrise) in each survey period (24 h). The horizontal bar denotes duration of observation of a given individual after its initial detection. Arrows indicate data from the same recaptured individual.

TABLE 1. Morphological characters ($\bar{x}\pm$SD, followed by ranges in parentheses) of male and female *Opisthotropis kikuzatoi*. Data from the present and previous studies (i.e., Toyama, 1983; Ota and Mori, 1985; OPBE, 1993) are incorporated.

Sex	n	SVL	TL	VT	SC
M	7	394.6±101.8	95.7±22.16	188.9±7.84	76.1±6.54
		(219–506)	(58–122)	(180–198)	(69–89)
F	7	354.4±100.3	89.1±26.72	185.1±7.54	74.0±9.06
		(231–480)	(58–125)	(176–196)	(58–89)

studies (Toyama, 1983; Ota and Mori, 1985; OPBE, 1993) and were compared between sexes (Table 1). In all these characters (SVL, TL, VT, and SC), no statistically significant differences were recognized between males and females (ANOVA, $P>0.05$). SVL and BW of the recaptured male were, respectively, 354 mm and 9.2 g at initial capture, and 357 mm and 9.1 g at recapture, showing no discernible growth during the period of nearly seven months.

The time of day at which the snakes were found varied from 0200 to 1405. From April to August all snakes were found from late morning to early afternoon, whereas all findings but one (for a female found at 0905 of 24 November) were made between midnight and sunrise during the period from September to March (Fig. 1).

SWT, SAT, and SWD in the present study varied from 16.3–27.2 C, 12.7–32.0 C, and 31–75 mm (Appendix 1), whereas WT, AT, and WD at capture ($\bar{x}\pm$SD, followed by ranges in parentheses) were 22.7±4.05 (16.4–28.5) C, 23.7±6.43 (14.5–31.1) C, and 34.0±29.73 (11–105) mm, respectively. BT of the snake at capture, 24.7±5.21 (16.4–29.2) C, was significantly correlated with both WT ($r^2=0.895$, $t=10.32$, $P<0.001$) and AT ($r^2=0.857$, $t=9.93$, $P<0.001$). BT was almost consistently higher than WT, and also substantially exceeded AT when AT was equivalent to or lower than 26.2 C. In contrast, BT was distinctly lower than AT when AT was 30.0 C or higher (Fig. 2). Regression lines for WT and BT during the warmer (i.e., April–September) and cooler seasons (November–March: see Appendix 1), BT= 0.215*WT+22.98 ($n=6$, $r^2=0.60$, $t=2.44$, $P<0.05$) and BT=1.397*WT-6.73 ($n=4$, $r^2=0.98$, $t=9.48$, $P<0.05$), respectively, significantly differed from each other in slope (ANCOVA: $F=55.6$, $P<0.001$),

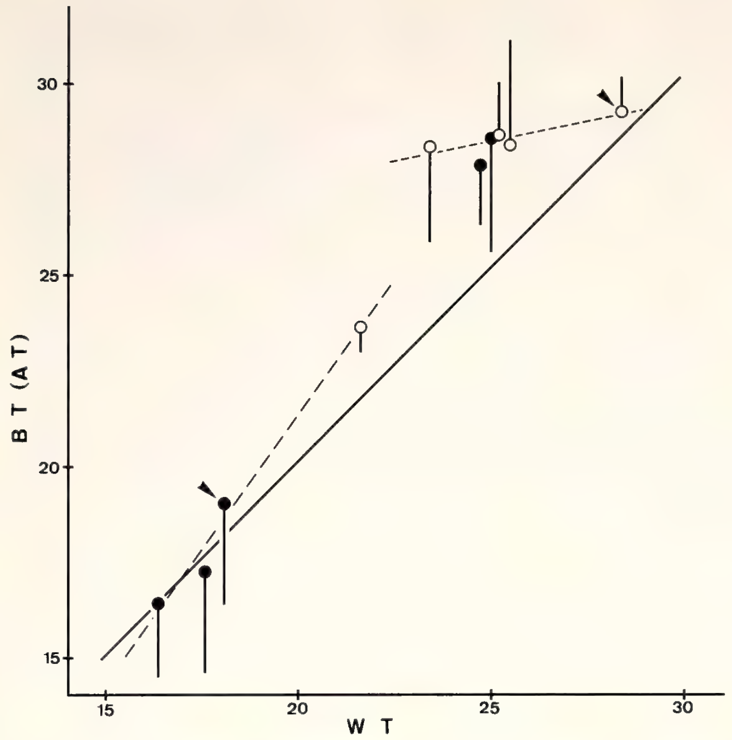

FIG. 2. Body temperatures of *O. kikuzatoi* (BT) in relation to water temperatures (WT) at capture. Open circles represent data obtained in the daytime, and closed circles at night. Arrows indicate data from the same recaptured individual. Vertical bars denote the differences of BT from air temperatures (AT) at capture. The solid line shows the location of BT=WT. The dotted line and dashed line represent regression lines for data obtained in the warmer (i.e., April-September, n=6) and the cooler seasons (November-March, n=4), respectively. See text for their equations.

but not in position (F=3.56, P>0.05).

Of the total of ten snakes examined (counting the recaptured male in duplicate), only one male, captured at 04:00 of 22 December (BT=17.2 C, WT=17.6 C, AT= 14.6 C, and WD=38 mm), had stomach contents, which consisted of three half-digested juveniles of the freshwater crab, *Candidiopotamon kumejimense*. In the study area, freshwater crabs of comparable sizes were seen in relatively high density from May to September, and in distinctly lower density from November to April (Appendix 2). For the five females, no oviductal eggs were detected by palpation.

DISCUSSION

A number of studies has been carried out on the seasonal activity patterns in snakes including some more or less aquatic species (e.g., Mushinsky et al [1980], Shine and Lambeck [1985], and other papers cited in Gibbons and Semlitsch [1987]). Most such studies are about species in almost aseasonal tropics, or at much higher latitudes where low temperatures have crucial effects upon the winter activity of ectotherms by forcing them into hibernation. In contrast, very little information is available regarding snakes in the subtropical regions (including the Ryukyu Archipelago), where seasonal climatic changes are prominent on the one hand, but winter temperature is not necessarily so low as to force ectotherms into hibernation on the other hand (e.g., Koba, 1962; Ota, 1994; Mori et al., 2002). The present results suggest that *Opisthotropis kikuzatoi* remains active almost throughout the year (Fig. 1), although the failure in finding even a single snake in the January and February surveys may reflect a drop in its activity during the middle–late winter when ambient temperatures are lowest (Appendix 1).

With respect to the daily activity pattern, several studies have also been conducted for aquatic snakes, leading to recognition of a few distinct patterns. Shine and Lambeck (1985), for example, clarified that the tropical acrochordid species, *Acrochordus arafurae*, is primarily nocturnal, whereas Voris and Karns (1996) and Karns et al. (2001) demonstrated that two tropical homalopsines, *Enhydris plumbea* and *E. enhydris*, are active both day and night. For the genus *Opisthotropis*, several Chinese and Southeast Asian species, such as *O. andersoni*, *O. kuatunensis*, *O. lateralis*, *O. latouchii*, and *O. typica*, are implicitly or explicitly referred to as nocturnal, whereas *O. baleatus* as diurnal (Pope, 1935; Zhong, 1984; Karsen et al., 1986; Mori, 1993; Zhao et al., 1998). It is, however, almost certain that all these statements were made on the basis of limited observations and thus are rather anecdotal. Only Mori and Nakachi (1994) attempted a quantitative approach to the daily activity pattern of the genus by videorecording and analyzing activities for two female *O. kikuzatoi* in captivity. The results

indicated that both females were most active in the morning and least so at night, and these authors thus suspected this species to be primarily diurnal unlike many other congeneric species (see above).

In the present study, however, five out of 11 observations of active snakes were made at night, although the remainder were in the daytime. Furthermore, one male, found active in the daytime at first capture, was recaptured after midnight (Fig. 1). These suggest that the daily activity pattern of *O. kikuzatoi* is more variable than was assumed by Mori and Nakachi (1994). Considering that captive observation by Mori and Nakachi (1994) was conducted from July to August, such an inconsistency between their result and the present observation may actually reflect seasonal variation in daily activity in this species.

Mori and Nakachi (1994) also reported that in the morning the two captive *O. kikuzatoi* were frequently active on the land portion in an otherwise water-filled cage. Observation of a snake escaping into the land vegetation in June (see above) confirms that *O. kikuzatoi* occasionally autonomously emerges from water and utilizes the land habitat.

Mushinsky et al. (1980) reported that some semiaquatic natricine snakes in a warm temperate area of the southern US tend to be nocturnal in summer and diurnal in winter. Such a pattern of seasonal variation in daily activity is also reported for a number of other temperate snakes, and is usually explained on the ground of behavioral adaptation to the seasonally varying thermal environment (Mushinsky et al., 1980; Gibbons and Semlitsch, 1987; Peterson et al., 1993). In this regard, the daily activity pattern of *O. kikuzatoi*, tending to be nocturnal in the cooler season and diurnal in the warmer season, looks puzzling (Figs. 1 and 2). Other factors, such as seasonally varying patterns of food availability, mating intensity, and predation pressure, may be responsible for such an apparently paradoxical pattern (see Gibbons and Semlitch [1987] for review of factors affecting activity patterns in snakes).

BT of *O. kikuzatoi* in the warmer season, mostly distinctly higher than WT irrespective of time of day at capture but with a less steep slope of the regression line, suggest the snake's motive thermoregulation, presumably by use of both sunlight (day) and heated ground surface (night). Also, the position of the regression line suggests that the snake prefers 29–30 C as an active body temperature (Fig. 2). Further studies are needed to verify each of the issues assumed above.

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As is mentioned in the text, handling of *Opisthotropis kikuzatoi* is strictly regulated by the laws of the Japanase National Government and of the Okinawa Prefectural Government. This research was carried out under official permissions from these governments.

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APPENDIX 1

Weather (Fi, fine; Cl, cloudy; Rn, rainy; lt: later; oc: occasionally), and minimum and maximum water temperatures (SWT, in C), air temperatures (SAT, in C) and water depths at a standard point (SWD, in mm) during each of the monthly survey. Time of day, at which each value was obtained, is given in parentheses.

Survey period	Weather	SWT		SAT		SWD	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1996.04.28 (09:00)–04.29 (08:30)	Fi (lt Cl)	17.9 (21:00)	19.0 (10:00–12:00)	20.1 (02:00)	26.3 (12:00)	36 (15:00–16:00)	40 (10:00)
1996.05.29 (13:00)–05.30 (12:30)	Fi	19.0 (06:00)	20.8 (12:00)	18.1 (04:00)	26.3 (12:00)	48 (11:00)	54 (06:00)
1996.06.23 (12:00)–06.24 (11:30)	Fi	23.4 (02:00–06:00)	24.5 (10:00)	26.1 (04:00)	31.9 (10:00)	42 (15:00)	49 (06:00)
1996.07.06 (13:00)–07.07 (12:30)	Fi	24.7 (00:00–06:00)	25.5 (10:00–12:00)	25.6 (22:00)	32.0 (12:00)	35 (14:00–17:00)	48 (04:00)
1996.08.26 (12:00)–08.27 (11:30)	Fi	23.9 (02:00–06:00)	25.8 (10:00)	22.6 (06:00)	30.7 (09:00–10:00)	37 (17:00)	45 (22:00)
1996.09.07 (16:00)–09.08 (15:30)	Fi (oc Cl)	24.8 (04:00)	27.2 (11:00–12:00)	25.5 (04:00)	30.3 (12:00–14:00)	31 (19:00)	41 (04:00–09:00)
1996.11.24 (13:00)–11.25 (12:30)	Rn (oc Cl)	21.6 (00:00–07:00)	21.9 (13:00–14:00)	20.6 (18:00)	23.2 (12:00)	47 (10:00, 12:00)	58 (20:00)
1996.12.21 (13:00)–12.22 (12:30)	Fi (oc Cl)	17.0 (07:00–08:00)	17.9 (14:00–15:00)	12.7 (08:00)	19.4 (14:00)	35 (18:00)	40 (07:00–11:00)
1997.01.18 (13:00)–01.19 (12:30)	Cl (oc Fi)	17.4 (02:00)	18.1 (13:00–15:00)	15.4 (06:00)	19.3 (13:00–14:00)	45 (06:00–07:00)	49 (16:00)
1997.02.15 (13:00)–02.16 (12:30)	Cl (oc Rn)	16.3 (11:00)	17.2 (16:00)	14.4 (09:00)	21.0 (14:00)	43 (13:00–17:00)	75 (20:00)
1997.03.22 (13:00)–03.23 (12:30)	Cl (oc Rn)	17.5 (06:00–08:00)	18.0 (14:00–15:00)	15.7 (10:00)	18.8 (14:00–15:00)	53 (06:00–12:00)	74 (13:00)

APPENDIX 2

Other animals observed in the stream during each of the monthly survey. Abbreviations are as follows. D, daytime. N, night. FCL, large freshwater crab. FCS, small freshwater crab. SS, small shrimp. MS, *Macrobrachium* sp. DL, dragonfly larva. FL, firefly larva. AM, *Anguilla marmorata*. BJ, *Buergeria japonica*. MO, *Microhyla ornata*. RC, *Rana catesbeiana*. AP, *Amphiesma pryeri*. +, ten or fewer individuals in maximum per census. ++, more than ten individuals in maximum per census.

		Animals										
Month*	D/N	FCL	FCS	SS	MS	DL	FL	AM	BJ	MO	RC	AP
April	D	+	+	+					+	+		
	N	+	+	++	+			+	++	+	+	
May	D	+	++	+	+				+			+
	N	+	++	++	+			+	+	+		
June	D	++	++	++					+			+
	N	++	++	++	+			+	++	+		+
July	D	++	++	++								
	N	++	++	++	++			+	+	+		
August	D	++	++	++	+							
	N	++	++	++	++			+	+	+		+
September	D	++	++	++	+							
	N	++	++	++	++			+	+			
November	D	+	+	+								
	N	+	+	+	+		+	+				
December	D	+	+	+								
	N	+	+	++	+				+			
January	D	+	+	++		+						
	N	+	+	++	+							
February	D	+	+	++								
	N	+	+	++	+			+				
March	D	+	+	++		+						
	N	+	+	++	+	+		+	+	+		

* See Appendix 1 for detailed period of each survey.

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Salinity and Other Abiotic Characteristics of Oviposition Sites of the Rhacophorid Frog, *Buergeria japonica*, in Coastal Habitat

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Abstract: Oviposition sites of *Buergeria japonica* were surveyed in portions of two streams near the coast on Okinawa Island, Ryukyu Archipelago, Japan. Twenty-five and 52 oviposition sites were found. At all these sites the salinity was constantly 2‰ or less despite occasional exposure of these portions to the sea breeze. The pH value at the sites, ranging from 7.7–10.2, never dropped into the acid range. There were no significant differences in water depth or distance from the bank at the oviposition sites between the two streams, despite the prominent differences in some topographic characters, such as maximum water depth and stream width.

Key words: Salinity; Oviposition site; Coastal habitat; Frogs; *Buergeria japonica*

Amphibians are susceptible to chemical factors of the ambient water throughout the aquatic embryonic and larval stages of their life cycles (Tyler-Jones et al., 1989). One of the most crucial chemical factors for their successful breeding and development is salinity, because in many amphibian species including those that have a high salinity tolerance in adults (e.g., *Rana cancrivora*: Gordon et al., 1961), the rate of egg survival or of metamorphosis falls remarkably even with a slight increase in salinity (Gordon and Tucker, 1965; Beebee, 1985; Uchiyama et al., 1990; Voigt, 1991). Nonetheless, some amphibians occur in potentially highly saline habitats, such as ponds and streams located within the reach of saltwater spray and tidal movements, and desert ponds where the salinity often remarkably increases with evaporation. For such frogs, it is essential to acquire high salinity tolerance during the embryonic and larval period, or otherwise, the particular ability to avoid breeding at highly saline sites. Indeed, Uchiyama et al. (1990) reported that in *R. cancrivora* living in a coastal marsh, spawning occurs only after heavy rainfall so as to allow eggs an early embryonic development under low salinity condition. For other frogs inhabiting potentially saline habitats, however, no studies have been made regarding their adaptation for successful reproduction.

Buergeria japonica is a ground-dwelling rhacophorid frog distributed on most islands of the Ryukyu Archipelago and on Taiwan. This frog is known to utilize various habitats including those near the seacoast, and its eggs are sometimes laid in the stream mouth no more than 100 m from the coastline (Maeda and Matsui, 1989). In the present study, I investigated salinity at oviposition sites of *B. japonica* in streams near the coast. I also describe several other abiotic characters for such oviposition sites of this frog.

MATERIALS AND METHODS

For the field survey, I chose two streams (streams 1 and 2), approximately 3.5 km apart from each other, in Kunigami Village in the northern part of Okinawa Island, Japan (26°50'N, 128°17'E). The portions surveyed were from the coastline up to approximately 100 m inland for stream 1 and from the coastline up to approximately 230 m inland for stream 2. Both streams usually flow on the surface of a sandy beach, directly entering the sea. Occasionally, however, they flow underground at the beach, leaving the surfaces dry. There was a little grass around the streams, and their bottoms were mainly composed of pebbles, sand, and pieces of dead coral.

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During the study periods (see below), standard air temperatures for streams 1 and 2 (measured at fixed points 1 m above the ground in shade) varied from 26.1 to 36.8 C and from 20.4 to 31.9 C, and the water temperatures ranged from 22.3 to 33.0 C and from 19.7 to 31.3 C, respectively. No other anuran species were observed.

Periods of the survey were from April to October 2001 for stream 1, and from April to September 2002 for stream 2. During these periods, I conducted route census almost everyday between 1200 and 1800 h by slowly walking along the portions of the streams, carefully searching for eggs laid. Census was not made on rainy days, because of the expected temporary lowering of salinity at oviposition sites. Eggs were usually attached to the substrate surface in masses. Thus, free single eggs, probably washed down from upper streams, were excluded from the analysis. Egg masses found were assumed to have been laid in the previous night. For each site at which an egg mass was located, I recorded the water salinity with a Salinity Refractometer (Atago., Hand Refractometer) and pH with a Quikchek (Orion Model 103), as well as water depth and distance from the bank. Maximum water depth was also measured at the deepest point along a transverse line to the stream, crossing the oviposition site. Widths of the portions of the streams surveyed were measured at 2.5 m intervals.

The salinity at oviposition sites was compared between the two streams by a χ^2-test, because values of this variable are integral numbers. Statistical analyses of the egg numbers and pH were made using Welch's t-test, because for either of these variables homogeneity of variance was not met between the two data sets. The other oviposition site characteristics (water depth and distance from the bank) and stream topography were compared using the Mann-Whitney U-test, because data deviated considerably from normal distribution. In all these tests the significance level was $P<0.05$.

RESULTS AND DISCUSSION

Twenty-five and 52 oviposition sites were detected in streams 1 and 2, and the mean numbers (±SD) of eggs found were 17.7±21.1 (range: 2–65) and 28.1±33.9 (2–181), respectively. These values showed no significant differences (Welch's t-test, $t=-1.65$, $df=69.9$, $P>0.05$).

Salinity was 2‰ or less at all these oviposition sites. No significant differences were found between the two streams (χ^2-test, $\chi^2=4.10$, $df=2$, $P>0.05$: Fig. 1). This suggests that even in a coastal habitat occasionally exposed to salty sea breeze the salinity at oviposition sites of *B. japonica* is not so high as to cause high mortality of eggs.

The pH values at oviposition sites were 8.5±0.4 (8.0–9.5) and 8.9±0.8 (7.7–10.2) in streams 1 and 2, respectively, and these significantly differed (Welch's t-test, $t=-2.4$, $df=44.2$, $P<0.05$). It has been reported that, in frogs, low pH (approximately 3.6–4.75) in ambient water interrupts embryonic development or produces abnormalities in the spine and tail of larvae (Gosner and Black, 1957; Freda and Dunson, 1985, 1986; Tyler-Jones et al., 1989). This suggests that in the present study area *B. japonica* do not suffer high embryonic mortality or larval abnormality from low pH.

The maximum water depth significantly differed between the two streams (25±20 mm [1–105 mm] in stream 1 and 47±29 mm [12–

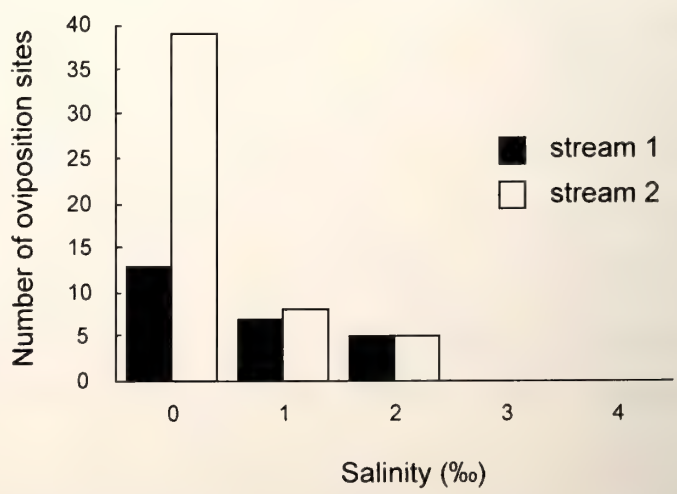

FIG. 1. Salinity at oviposition sites of *Buergeria japonica* in streams 1 and 2.

148 mm] in stream 2: Mann-Whitney U-test, Z=–3.84, P<0.001). Nevertheless, the water depth at oviposition sites showed no significant difference between sites (12±8 mm [4–30 mm] in stream 1; 13±8 mm [0–34 mm] in stream 2: Mann-Whitney U-test, Z=–0.49, P>0.05; Fig. 2). Likewise, although stream 1 (791±501 mm [260–2250 mm]) was significantly narrower than stream 2 (4283±2231 mm [760–8690 mm]: Mann-Whitney U-test, Z=–6.63, P<0.001), distance from oviposition sites to the bank did not significantly differ between sites (197±156 mm [30–490 mm] in stream 1; 182±285 mm [0–2000 mm] in stream 2: Mann-Whitney U-test, Z=–0.92, P>0.05: Fig. 3).

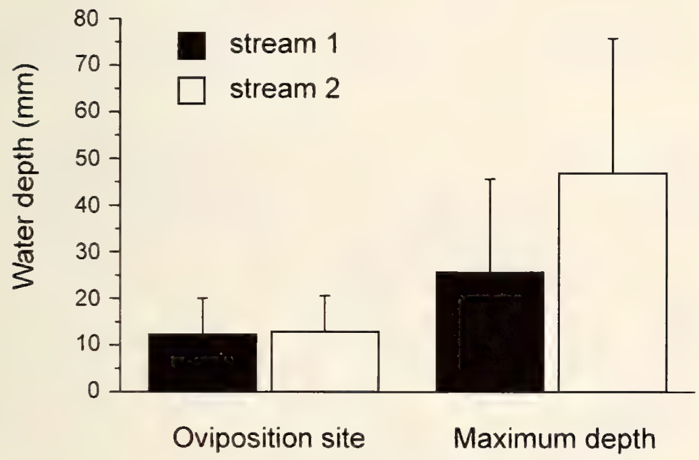

FIG. 2. Comparison of the water depth at oviposition sites of *Buergeria japonica* (left) and the maximum water depth (right: see text for definition: both in mm) between streams 1 and 2. Vertical bars denote 1 SD.

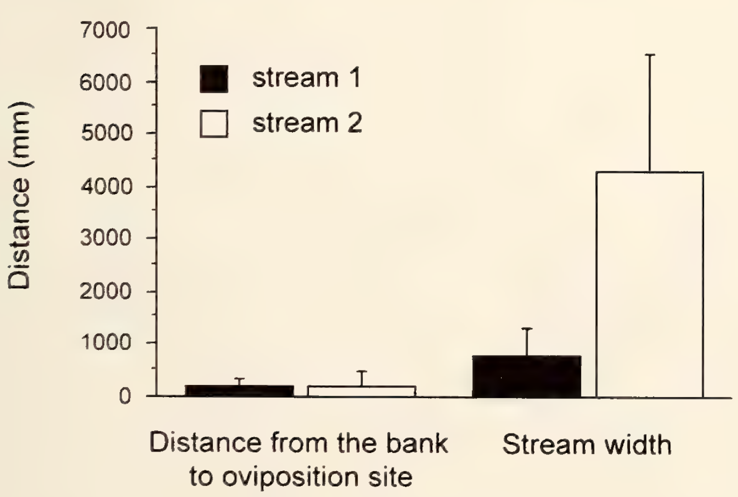

FIG. 3. Comparison of the distance from the nearest bank to oviposition sites of *Buergeria japonica* (left) and the stream width (right: see text for definition: both in mm) between streams 1 and 2. Vertical bars denote 1 SD.

These results may suggest that this frog chooses oviposition sites at least partially on the basis of water depth and distance from the bank. However, these issues definitely need further careful verification, particularly by considering correlation among these topographic parameters and such chemical parameters as the salinity and pH of the water.

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Current Herpetology 23(2): 85–86, December 2004

ERRATUM: "Two Unknown Arboreal Frogs (Genus *Platypelis*) Described from the Rainforests of Northeastern Madagascar (Microhylidae: Cophylinae)", by F. Andreone, D. B. Fenolio, and M. E. Walvoord, *CURRENT HERPETOLOGY* 22(2): 91–100, 2003

A recent paper describing two new *Platypelis* species (*P. tetra* and *P. mavomavo*) by Andreone et al. (2003: see above) was accompanied by three sets of erroneous drawings with misleading captions, giving incorrect and confusing information on these species. Here the authors provide corrected drawings and figure numeration. One major correction that was not in accord with what was reported in the text is that the pupils of both species are horizontal, not vertical. Furthermore, the tympanum, not shown in either of the original drawings, is actually visible in both species. The foot in the former Fig. 6C, attributed to *P. mavomavo*, in reality showed the foot of *P. tetra*. In the new figure, the foot is correctly portrayed, clearly showing the species' typical length reduction in toes I–III and the presence of enlarged, triangular toe pads. Finally, the former Fig. 5 caption actually corresponded to Fig. 6 and vice versa. The new Fig. 5 below has the right caption referring to the ventral view of the holotype of *P. mavomavo*, while Fig. 6 has the complete caption for the drawings of the head, hand, and foot of *P. mavomavo*.

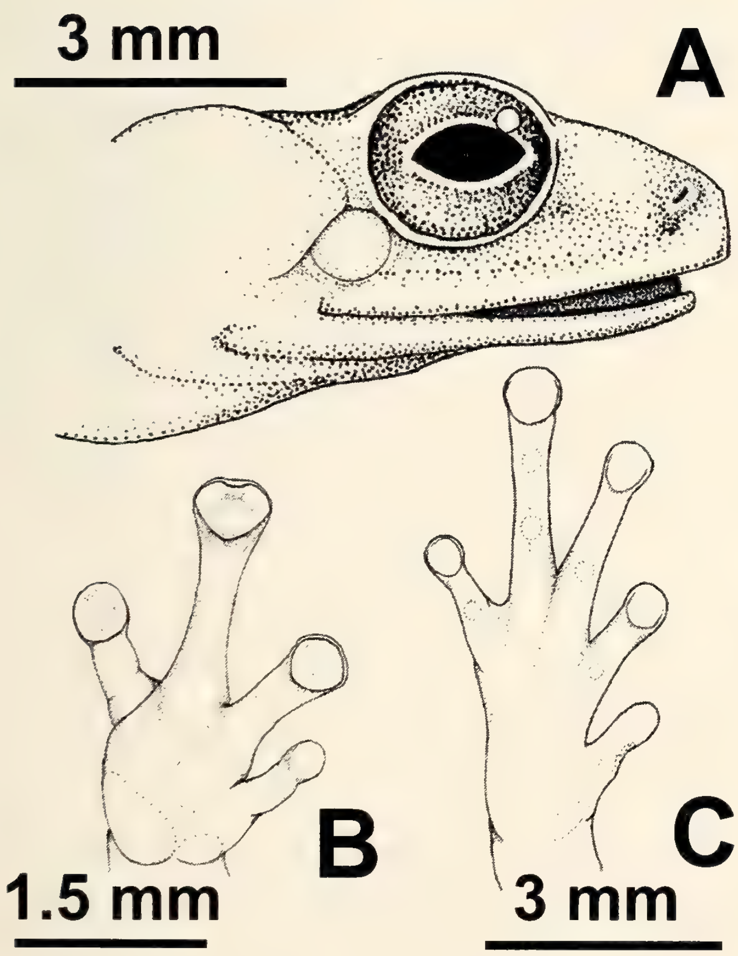

FIG. 2. Particular of the head (A), hand (B), and foot (C) of the holotype of *Platypelis tetra* (male, MRSN A2174).

FIG. 5. *Platypelis mavomavo*. Holotype (MRSN A2435), ventral view.

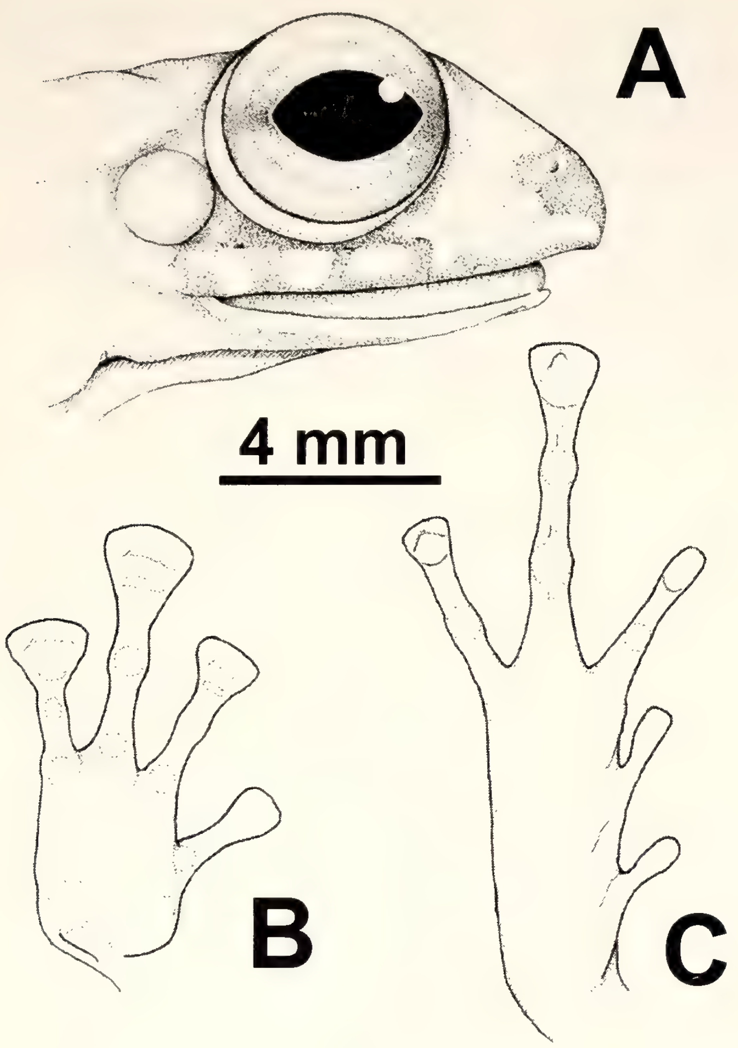

FIG. 6. Particular of the head (A), hand (B), and foot (C) of the holotype of *Platypelis mavomavo* (male, MRSN A2435).

Current Herpetology 23(2): 87–90, December 2004

Titles of Papers Presented at the 43rd Annual Meeting of the Herpetological Society of Japan (23–24 October 2004 at Kyoto University, Kyoto)

Abstracts (in Japanese) will appear in Bulletin of the Herpetological Society of Japan, Vol. 2005, No. 1.

SPECIAL SESSION

1. Adventures of a "Snake Boy". Richard C. Goris
2. Making developmental tables for amphibians. Hisaaki Iwasawa

GENERAL SESSION

[Salamanders and newts]

1. Distribution and status of the Japanese giant salamander, *Andrias japonicus* in Tottori Prefecture. Sumio Okada and Tamami Okada
2. Movement patterns of the Japanese giant salamander, *Andrias japonicus*, in the Hino River, Tottori Prefecture. Tamami Okada and Sumio Okada
3. Reproduction of *Hynobius nebulosus* in an artificial pond in Kyoto. Yuko Watanabe, Masafumi Matsui, and Kanto Nishikawa
4. Groups of *Hynobius nebulosus* in the Chugoku District. Hiroshi Okawa, Taeko Utsunomiya, and Takashi Okuno
5. Habitat use by the Siberian salamander *Salamandrella keyserlingii* distributed in Kushiro Marsh, Hokkaido. Takanori Sato, Shigehiro Nakabayashi, Nobuyuki Narumi, and Takehito Ueda
6. Use of passive integrated transpoder (PIT) tags to individually identify *Hynobius kimurae*. Yasuchika Misawa and Masafumi Matsui
7. Molecular cytogenetic analysis of the highly repetitive DNA in six hynobiid salamanders, with comments on their phylogenetic relationships. Masaki Kuro-o, Hiromi Takahashi, Eriko Inada, Yoko Hasegawa, Guanfu Wu, Xiaomao Zeng, Chikako Ikebe, and Sei-ichi Kohno
8. Ag-NOR sites in chromosomes of four *Hynobius* species and FISH analysis of major rRNA gene loci in *H. nigrescens*. Chikako Ikebe, Aihua Cui, Masaki Kuro-o, Souichiro Kubota, and Sei-ichi Kohno
9. Repeated DNA sequence of the giant salamander—The Xba repeat rich in palindromes. Kosuke Sako, Kei Okamoto, Kenichiro Mizuno, Shunsuke Tanigawa, Yoshinobu Suizu, Takahiro Nakanishi, Kyosuke Kawasumi, Yuki Harada, Tatsumi Morita, Masami Akagawa, Tomoaki Sawaguchi, Yukio Fukumoto, Kazushi Kuwabara, Kazuhide Ashikaga, and Ikuo Miura
10. The mitochondrial DNA of giant salamander—Its gene organization and evolutionary rate of base substitution. Kei Okamoto, Kosuke Sako, Kenichiro Mizuno, Shunsuke Tanigawa, Yoshinobu Suizu, Takahiro Nakanishi, Kyosuke Kawasumi, Yuki Harada, Tatsumi Morita, Masami Akagawa, Tomoaki Sawaguchi, Yukio Fukumoto, Kazushi Kuwabara, Kazuhide Ashikaga, and Ikuo Miura
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18. Seasonal fluctuation of prey items of the newt, *Cynops pyrrhogaster*, in the Yachidaira Marsh. Sadao Ihara

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51. Reproductive ecology of female Asian yellow pond turtles, *Mauremys mutica kami*, investigated by X-ray photography. Takashi Yabe
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78. First record of the himehabu on Kakeroma Island in the Amami Islands, Japan. Korebumi Minakami and Shigeo Takase
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MOODY, S. M. 1980. Phylogenetic and Historical Biogeographical Relationships of the Genera in the Family Agamidae (Reptilia: Lacertilia). Unpublished doctoral dissertation. University of Michigan, Ann Arbor.

TAKENAKA, T. 2000. Extinction of the naturalized freshwater turtle in Chichijima-Island

of Ogasawara (Bonin) Islands, South Japan. Bull. Herpetol. Soc. Japan 2000: 4-7. (in Japanese with English abstract)
ZHAO, E. AND K. ADLER. 1993. Herpetology of China. Contribution to Herpetology, 10. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

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APPENDIX

Specimens examined

Catalogue numbers of specimens deposited in the zoological collection of Kyoto University Museum are preceded by KUZ. The other acronyms are those suggested by Leviton et al. (1985).

Geoemyda japonica: Okinawajima, Okinawa Pref., Japan, KUZ R36720, NSMT H02083-02086; Kumejima, Okinawa Pref., Japan, KUZ R36721, OMNH-R3334. G. spengleri: Vietnam, NSMT H9999, ...

Use major headings with consecutive Roman numerals, APPENDIX I, APPENDIX II, APPENDIX III, APPENDIX IV, etc., when two or more appendices are needed.

Tables

Each table should be typed, double-spaced, **on a separate sheet. Its appropriate position in the text should be marked in the right-hand margin** (usually at the place where the table is first mentioned). The legend for a table should follow the table number (e.g., TABLE 1. Measurements of specimens of ......) and **should be on the same pages as the table**. Within

the table, only the initial letter of the first word should be capitalized (e.g., "Adult males"). **Ruled lines should be avoided**. Footnotes (indicated by symbols *, or, *1, *2, *3, etc.) may follow a table when detailed information is needed.

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Figures larger than 28×21 cm cannot be accepted. When several drawings or photographs are to be reproduced as one figure, **they should be mounted on cardboard in the desired arrangement and each of them should be lettered** (A, B, C, etc.). Each figure should be carefully planned to accommodate reduction to a final width of one or two columns in *Current Herpetology*; after reduction, lettering should be≥1.5 mm high and decimals should be visible. Include a scale of distance or dimension where appropriate. Figure legends, each beginning with a fully capitalized abbreviation and a consecutive number (e.g., FIG. 1. Dorsal (A) and lateral (B) views of holotype of ......), should be typed in double space and **grouped together on a separate sheet** with one line of space between them. **Denote in the right-hand margin of the text where each figure should be printed** (usually at the first mention of a figure). In the text, "Figure" should be also abbreviated, but with only the initial letter capitalized (e.g., Fig. 1) except when beginning a sentence. When original figures are sent to the Managing Editor after the acceptance of a manuscript (see above), the back of each figure should be labeled with the author's name, the figure number, and desired size of final reproduction (one column or two columns are the only options).

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Abbreviations

Weights and measures follow the International System of Units (SI), and such abbreviations should be used throughout the manuscript. Abbreviations used in *Current Herpetology* without being defined at first usage include:

$\bar{x}$ (mean), n (sample size), N (chromosome number, but see below for the use to refer to "north latitude"), no. (number), yr (year[s]), mo (month[s]), wk (week[s]), h (hour[s]), min (minute[s]), s (second[s]), P (probability), df (degrees of freedom), SD (standard deviation), SE (standard error), NS (not significant), l (liter), kg (kilogram), g (gram), m (meter), cm (centimeter), mm (millimeter), µm (micron), C (degrees Celsius, not °C), asl (above sea level; given as, e.g., 100 m asl), °, ', and " (degrees, minutes, and seconds in geography, respectively), N, S, E, and W (north and south latitudes, and east and west longitudes, respectively, but only when preceded by values with appropriate geographical units; e.g., 15°25'N, 121°43'E).

In figures and tables, names of months can be abbreviated as: Jan (January), Feb (February),

Mar (March), Apr (April), May (May), Jun (June), Jul (July), Aug (August), Sep (September), Oct (October), Nov (November), Dec (December). In the text, however, they should be fully spelled.

Other abbreviations, such as SVL (snout-vent length), Myr (million years), and MyrBP (million years before present), can also be used, but **define them at first usage**. Do not use symbols, ♂ and ♀, for male and female, respectively.

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